FORM PTO-1390 (REV. 11-2000) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: REG 670A-US

U.S APPLICATION NO. (If known, see 37 CFR 1 5): Not Yet Known 09/868677

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/30900	23 December 1999	23 December 1998

TITLE OF INVENTION
METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

APPLICANT(S) FOR DO/EO/US
Samuel J. Davis, Nicholas W. Gale, George D. Yancopoulos, Neil Stahl

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [X] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [X] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information: An unexecuted Declaration and Power of Attorney

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/868677	PCT/US99/30900	REG 670A-US

21. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 710.
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	77 - 20 =	57	x $18.00	$ 1,026.
Independent claims	6 - 3 =	3	x $80.00	$ 240.
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$ 270.
TOTAL OF ABOVE CALCULATIONS =				2,246.
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$
SUBTOTAL =				$ 2,246.
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 2,246.
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+	$
TOTAL FEES ENCLOSED =				$ 2,246.
			Amount to be refunded:	$
			charged:	$

a. [X] A check in the amount of $ 2,246. to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 18-0650 . A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.
Linda O. Palladino
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591

Linda O. Palladino
SIGNATURE

Linda O. Palladino
NAME

45,636
REGISTRATION NUMBER

Att. Dkt. No. - REG 670A-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Davis, S., et al.		
U.S. Serial No.:	Not yet known	**Examiner:**	Not yet known
Filing Date:	Filed herewith	**Group Art Unit:**	Not yet known

Title: **METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS**

June 20, 2001

Commissioner of Patents and Trademarks
United States Patent and Trademark Office
Washington, DC 20231

SIR:

PRELIMINARY AMENDMENT

This paper is submitted in connection with the above-identified application, which is a U.S. National Stage Application of PCT International Application No. PCT/US99/30900, filed December 23, 1999, which claims priority to U.S. Provisional Application No. 60/113,387, filed December 23, 1998, now abandoned. Prior to examination of the application on the merits, Applicants respectfully request entry of the following amendments to the claims and specification:

In the claims:

Please cancel claims 1-41 without prejudice and replace with new claims 42-88 as follows:

--42. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

43. (New) The isolated nucleic acid molecule of claim 42, wherein the receptor binding domains of the first and second subunits are the receptor binding domain from the same ligand.

44. (New) The isolated nucleic acid molecule of claim 42, wherein the receptor binding domain of the first subunit is a receptor binding domain derived from a different ligand then the receptor binding domain of the second subunit.

45. (New) The isolated nucleic acid molecule of claim 43, wherein the receptor binding domain of the first and second subunit is the fibrinogen domain of angiopoietin-1.

46. (New) The isolated nucleic acid molecule of claim 43, wherein the receptor binding domain of the first and second subunit is the fibrinogen domain of angiopoietin-2.

47. (New) The isolated nucleic acid molecule of claim 44, wherein the receptor binding domain of the first subunit is the fibrinogen domain if angiopoietin-1 and the receptor binding domain of the second subunit is the fibrinogen domain of angiopoietin-2.

48. (New) The isolated nucleic acid molecule of claim 44, wherein the receptor binding domain of the first subunit is the fibrinogen domain if angiopoietin-2 and the receptor binding domain of the second subunit is the fibrinogen domain of angiopoietin-1.

49. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of angiopoietin-1, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of angiopoietin-1.

50. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of angiopoietin-2, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of angiopoietin-2.

51. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

52. (New) An isolated nucleic acid molecule encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of a receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, and the multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

53. (New) The isolated nucleic acid molecule of claim 43, wherein the ligand is selected from the group consisting of the EPH family of ligands.

54. (New) The isolated nucleic acid molecule of claim 44, wherein the ligands are selected from the group consisting of the EPH family of ligands.

55. (New) The isolated nucleic acid molecule of claim 42, wherein the multimerizing component comprises an immunoglobulin derived domain.

56. (New) The isolated nucleic acid molecule of claim 43, wherein the multimerizing component comprises an immunoglobulin derived domain.

57. (New) The isolated nucleic acid molecule of claim 44, wherein the multimerizing component comprises an immunoglobulin derived domain.

58. (New) The isolated nucleic acid molecule of claim 55, 56, or 57, wherein the immunoglobulin derived domain is selected from the group consisting of the constant region domain of IgG, Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

59. (New) A fusion polypeptide encoded by the isolated nucleic acid molecule of claims 42, 43, or 44.

60. (New) The fusion polypeptide of claim 59, wherein the fusion polypeptide is multimerized.

61. (New) A composition comprising the multimerized fusion polypeptide of claim 60.

62. (New) The composition of claim 61, wherein the multimer is a dimer.

63. (New) A vector which comprises the isolated nucleic acid molecule of claims 42, 43, or 44.

64. (New) An expression vector comprising a isolated nucleic acid molecule of claims 42, 43, or 44, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

65. (New) A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 64, in a suitable host cell.

66. (New) The host-vector system of claim 65, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

67. (New) The host-vector system of claim 66, wherein the suitable host cell is E. coli.

68. (New) The host-vector system of claim 66, wherein the suitable host cell is a COS cell.

69. (New) The host-vector system of claim 66, wherein the suitable host cell is a CHO cell.

70. (New) A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 66, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

71. (New) An isolated nucleic acid molecule encoding a fusion polypeptide, wherein the fusion polypeptide comprises more than one copy of a receptor binding domain of a ligand, each copy fused in tandem, and wherein either the N-terminal or the C-terminal ends of the tandem receptor binding domains is fused to a multimerizing component.

72. (New) The isolated nucleic acid molecule of claim 71, wherein the receptor binding domains are fused contiguously.

73. (New) The isolated nucleic acid molecule of claim 71 or 72, wherein the ligand is not a member of the EPH family of ligands.

74. (New) The isolated nucleic acid molecule of claim 71 or 72, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

75. (New) The isolated nucleic acid molecule of claim 71 or 72, wherein the multimerizing component comprises an immunoglobulin derived domain.

76. (New) The isolated nucleic acid molecule of claim 75, wherein the immunoglobulin derived domain is selected from the group consisting of the constant region domain of IgG, the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

77. (New) A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 71.

78. (New) The fusion polypeptide of claim 77, wherein the fusion polypeptide is multimerized.

79. (New) A composition comprising the multimerized fusion polypeptide of claim 78.

80. (New) The composition of claim 79, wherein the multimerized fusion polypeptide is a dimer.

81. (New) A vector which comprises the isolated nucleic acid molecule of claim 71.

82. (New) An expression vector comprising a nucleic acid molecule of claim 71, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

83. (New) A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 82, in a suitable host cell.

84. (New) The host-vector system of claim 83, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

85. (New) The host-vector system of claim 84, wherein the suitable host cell is E. coli.

86. (New) The host-vector system of claim 84, wherein the suitable host cell is a COS cell.

87. (New) The host-vector system of claim 84, wherein the suitable host cell is a CHO cell.

88. (New) A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 83, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.--

Support for new claims 42-88 can be found throughout the specification.

In the specification

On page 1, line 3, before "This", please insert the following claim priority data: This application is a U.S. National Stage Application of PCT International Application No. PCT/US99/30900, filed December 23, 1999, which claims priority to U.S. Provisional Application No. 60/113,387, filed December 23, 1998, now abandoned.

REMARKS

Attached herewith as Exhibit A are marked up copies of the amended pages and as Exhibit B are substitute sheets of the amended pages.

Applicants have canceled claims 1-41 and replaced them with new claims 42-88. Therefore, Applicants will consider new claims 42-88 for the purpose of calculating the filing fees associated with the U.S. National Stage Application filed concurrently herewith. No fee is deemed necessary in connection with filing this Preliminary Amendment. However, if any fee is deemed necessary,

authorization is hereby given to charge the amount of any such fee to Deposit Account No. 18-0650.

Respectfully submitted,

Gail M. Kempler
Reg. No. 32,143
Joseph M. Sorrentino
Reg. No. 32,598
Attorneys for Applicant
Linda O. Palladino
Reg. No. 45,636
Patent Agent for Applicant
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
(914) 345-7400

METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

This application is a U.S. National Stage application of PCT International application No. PCT/US99/30900, filed December 23, 1999, which ~~This application~~ claims priority of U.S. Application No. 60/113,387, filed December 23, 1998. Throughout this application, various publications are cited. The disclosures of each and all of those publications are hereby incorporated by reference in their entireties into this application.

INTRODUCTION

The present invention provides for novel methods for producing novel fusion polypeptide ligands that have enhanced biological activity as compared to the polypeptide ligands in their native form. The invention also provides for nucleic acids useful for producing biologically active fusion polypeptide ligands, and the fusion polypeptide ligands themselves.

BACKGROUND OF THE INVENTION

The ability of polypeptide ligands to bind cells and thereby elicit a phenotypic response such as cell growth, survival or differentiation is often mediated through transmembrane tyrosine kinases. The extracellular portion of each receptor tyrosine kinase (RTK) is generally the most distinctive portion of the molecule, as it provides the protein with its ligand-recognizing characteristic. Binding of a ligand to the extracellular domain results in signal transduction via an intracellular tyrosine kinase catalytic domain which transmits a biological signal to intracellular target proteins. The particular array of sequence motifs of this cytoplasmic, catalytic domain determines its access to potential kinase substrates (Mohammadi, et al.,1990, Mol. Cell. Biol., 11: 5068-5078; Fantl, et al., 1992, Cell, 69:413-413).

RTKs appear to undergo dimerization or some related conformational change following ligand binding (Schlessinger, J., 1988, Trend Biochem. Sci.

METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

This application is a U.S. National Stage Application of PCT International Application No. PCT/US99/30900, filed December 23, 199, which claims priority of U.S. Application No. 60/113,387, filed December 23, 1998. Throughout this application, various publications are cited. The disclosures of each and all of those publications are hereby incorporated by reference in their entireties into this application.

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METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

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13:443-447; Ullrich and Schlessinger, 1990, Cell, 61:203-212; Schlessinger and Ullrich, 1992, Neuron 9:383-391); molecular interactions between dimerizing cytoplasmic domains lead to activation of kinase function. In some instances, such as the growth factor platelet derived growth factor (PDGF), the ligand is a dimer that binds two receptor molecules (Hart, et al. , 1988, Science, 240: 1529-1531; Heldin, 1989, J. Biol. Chem. 264:8905-8912) while, for example, in the case of EGF, the ligand is a monomer (Weber, et al., 1984, J. Biol. Chem., 259:14631-14636).

Throughout the history of the biotechnology industry, many novel genes and associated proteins have been identified by virtue of their sequence homology with known genes. Many such proteins are purported to be receptors, but since their cognate ligands have not been identified, they are referred to as orphan receptors. The screening of many of these orphan receptors often leads to the identification of ligands that are capable of binding to the receptor, although the binding is often not associated with activation of any intracellular kinases or any other phenotypic change. Such was the case for members of the Eph receptor family. For sake of clarity, applicants incorporate by reference herein a letter cited as Eph Nomenclature Committee, 1997, published in Cell vol. 90: 403-403 (1997) which sets forth a nomenclature for the Eph Receptor and Eph Ligand Families.

Little, if any, biological activity had been observed in response to binding of a ligand to an Eph family receptor prior to the discovery as set forth in U.S. Patent No. 5,747,033 issued May 5, 1998. That patent describes the concept of "clustering" whereby the soluble domains of ligands were combined to create multimers capable of activating the cognate receptors. Applicants have now extended the concept of clustering to additional ligands outside the Eph family, for example, the Tie-2 receptor ligands known as the angiopoietins, and have also discovered that this method for production of homogeneous forms of clustered ligands is broadly applicable to improve

the affinity and/or increase the activity of a ligand as compared to the native form of the ligand.

Angiopoietin-1 (Ang) is one of two known ligands for the Tie-2 receptor and has been shown to be an agonist for Tie-2 (Davis, et al, 1996, Cell 87:1161-1169), whereas the second known ligand, angiopoietin-2, has been shown to be a naturally occurring antagonist of the Tie-2 receptor (Maisonpierre, et al., 1997, Science 277:55-60). Ang1* is a mutant form of angiopoietin-1 that comprises the N-terminal domain of angiopoietin-2 fused to the coiled-coil domain and the fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. Ang1* has been shown to be a potent agonist for the Tie-2 receptor.

Experiments with mutants of angiopoietin-1 and angiopoietin-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions of, for example Ang-1-FD-Fc, (i.e., the fibrinogen domain of Ang-1 fused to an Fc domain), can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD (dimerization occurs due to the interaction between the Fc components of adjacent molecules). However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD (i.e., the fibrinogen domain of Ang-2) were designed that were intrinsically more highly clustered.

SUMMARY OF THE INVENTION

The present invention provides for novel, biologically active, soluble forms of polypeptide ligands that bind to receptors on cells. Such polypeptide ligands are useful in promoting a differential function and/or influencing the phenotype, such as growth and/or proliferation, of receptor-bearing

cells. The invention also provides for nucleic acids encoding such polypeptide ligands, and both prokaryotic and eukaryotic expression systems for producing such polypeptide ligands. According to the invention, soluble forms of the polypeptide ligands described herein may be used to promote biological responses in receptor-expressing cells. In particular, a general method is described herein which produces fusion polypeptide ligands that may then be clustered, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

DESCRIPTION OF THE FIGURES

Figure 1A-1E - Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-FD-Fc.

Figure 2A-2E - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-FD-Fc.

Figure 3A-3E - Nucleic acid sequence and deduced amino acid sequence of Ang-1-FD-Fc-FD.

Figure 4A-4E - Nucleic acid sequence and deduced amino acid sequence of Ang-2-FD-Fc-FD.

Figure 5 - Molecular Weight Analysis of Ang-1-FD-Fc-FD protein. SDS PAGE analyses showing a band running at about 210kD under non-reducing conditions (lane 3) and a band running at about 85kD under reducing conditions (lane 7).

Figure 6 - Light scatter analysis to confirm the molecular weight of Ang-1-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is homogenous.

Figure 7 - Molecular Weight Analysis of Ang-2-FD-Fc-FD. SDS PAGE analyses showing a band running at about 200kD under non-reducing conditions (lanes 7 and 8) and a band running at about 88kD under reducing conditions (lanes 3 and 4).

Figure 8 - Light scatter analysis to confirm the molecular weight of Ang-2-FD-Fc-FD and to determine whether or not the protein is a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent is monitored with an on line light scattering detector and a refractive index and/or a UV detector. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) is used to calculate the protein concentration. The molecular weight of protein is then calculated from the angular dependence of light scattering. The molecular weight of the dimeric protein appears to be approximately 171kD and presence of a single peak implies that the

protein solution is homogenous.

Figure 9 - Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926.

Figure 10 - Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

Figure 11 - Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells. In a standard phosphorylation assay, at a 20 fold molar excess, angiopoietin-2 is not able to reduce the Ang1*-mediated phosphorylation level to 50%. This result, coupled with the results described in Figure 10 implies that Ang-2-FD-Fc-FD is a more potent inhibitor of Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.

Figure 12 - Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10.

Figure 13 - Ability of angiopoietin-2 to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells. In a standard phosphorylation assay, it is shown that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at

blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11.

Figure 14A-14E - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B1-Ephrin-B1-Fc.

Figure 15A-15E - Nucleic acid sequence and deduced amino acid sequence of Ephrin-B2-Ephrin-B2-Fc.

Figure 16 - Comparison of Ephrin-B1-Fc, Ephrin-B1-Ephrin-B1-Fc, Ephrin-B2-Fc and Ephrin-B2-Ephrin-B2-Fc in standard EphB2 phosphorylation assays. COS cells were serum-starved and then left untreated (UT), lane 1, or were treated with unclustered and clustered Ephrin-B1-Fc (Efn-B1), lanes 2 and 3. COS cells were also treated with unclustered and clustered Ephrin-B1-Ephrin-B1-Fc (Efn-B1 DD), lanes 4 and 5. In addition cells were likewise treated with unclustered and clustered Ephrin-B2-Fc (Efn-B2), lanes 6 and 7 and with unclustered and clustered Ephrin-B2-Ephrin-B2-Fc (Efn-B2 DD), lanes 8 and 9. The extent of EphB2 phosphorylation was assessed by anti-phosphotyrosine western blotting (upper panels) and the relative amounts of EphB2 in each lane was determined by anti-EphB2 western blotting (lower panels).

Figure 17 - Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were stimulated with 0.4 μg/ml Ang1* or 0.2 μg/ml or 0.4 μg/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells.

Figure 18 - Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells. EAhy926 cells were treated with 0.2

μg/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 μg/ml, 4 μg/ml, 8 μg/ml or 16 μg/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. Ang-2-FD-Fc-FD is able to block or stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

DETAILED DESCRIPTION OF THE INVENTION

As described in greater detail below, applicants have discovered a method for "clustering" polypeptide ligands, which functions to make otherwise inactive soluble polypeptide ligands biologically active, or which enhances the biological activity of polypeptide ligands that, absent such clustering, would have lower levels of biological activity. This method may be used to cluster a plurality of (more than one) receptor binding domains from any ligand which has improved affinity and/or increased activity (i.e. signaling ability) when clustered as compared to the native form of the ligand.

The present invention provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

In one embodiment of the invention, the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand. The first and second subunits may each have one or more than one copy of the receptor binding domain of the ligand. In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. Alternatively, the

receptor binding domain is from a ligand selected from the group consisting of the EPH family of ligands (i.e., the ephrins).

In another embodiment of the invention, the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit. For example, the first subunit may comprise the receptor binding domain of an angiopoietin and the second subunit may comprise the receptor binding domain of vascular endothelial growth factor (VEGF). Alternatively, the first subunit may comprise the receptor binding domain of VEGF and the second subunit may comprise the receptor binding domain an angiopoietin. Still further, the first and second subunits may each have one or more than one copy of the receptor binding domain of their respective ligand.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(ΔC1).

The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996

and include S. cerevisiae repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) 103:321-326); the S. cerevisiae type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. 5:3381-3390); the S. calsbergensis alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene 36:333-340); and the Neurospora crassa ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. 262:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); the S. cerevisiae SUC2 gene (Carlson et al., 1983, Mol. Cell. Biol. 3:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS or CHO cell.

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system.

The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The present invention also provides for a nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component. In one embodiment of the invention, the receptor binding domains are fused contiguously. In another embodiment of the invention, the receptor binding domains are from a ligand that is not a member of the EPH family of ligands (i.e., not an ephrin). In specific embodiments of the invention, the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2. In an alternative embodiment, the receptor binding domain is from vascular endothelial growth factor (VEGF). In another embodiment, the receptor binding domain is from an ephrin.

By "receptor binding domain" what is meant is the minimal portion of the ligand that is necessary to bind its receptor.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(ΔC1).

The present invention also provides for fusion polypeptides encoded by the nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are described in European Patent Application of ZymoGenetics, Inc., Publication No. EP 0 721 983 A1 published 17 July 1996 and include S. cerevisiae repressible acid phosphatase (Mizunaga et al., 1988, J. Biochem. (Tokyo) 103:321-326); the S. cerevisiae type 1 killer preprotoxin (Sturley et al., 1986, EMBO J. 5:3381-3390); the S. calsbergensis alpha galactosidase melibiase (Sumner-Smith, et al., 1985, Gene 36:333-340); and the Neurospora crassa ornithine decarboxylase (Digangi, et al., 1987, J. Biol. Chem. 262:7889-7893). Sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); the S. cerevisiae SUC2 gene (Carlson et al., 1983, Mol. Cell. Biol. 3:439-447); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is

operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS or CHO cell.

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system. The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The Examples describe the preparation of novel polypeptide ligands that comprise a receptor binding domain of a member of the Eph (Eph transmembrane tyrosine kinase family ligands) family of ligands or of a

member of the angiopoietin family of ligands that can bind the Tie-2 receptor.

For a description of novel Eph family ligands, methods of making and using them, as well as the sequences of EHK-1L, B61 and ELK-L, together with a description of a method of enhancing the biological activity of EPH family ligands by clustering them, applicants refer to U.S. Patent No. 5,747,033 issued on May 5, 1998 which is hereby incorporated by reference in its entirety. Applicants further refer to International Application PCT/US93/10879, published as WO 94/11020 on May 26, 1994; and International Application PCT/US96/17201 published as WO 97/15667 entitled "Biologically Active EPH Family Ligands" each of which is hereby incorporated by reference in its entirety.

As has been previously reported, a family of ligands for the TIE-2 receptor has been discovered and named the Angiopoietins. This family, consisting of TIE-2 ligand 1 (Ang-1); TIE-2 ligand 2 (Ang-2); TIE ligand 3 (Ang-3); and TIE ligand 4 (Ang-4) has been extensively characterized. For a description of the cloning, sequencing and characterization of the angiopoietins, as well as for methods of making and uses thereof, including the production and characterization of modified and chimeric ligands thereof, reference is hereby made to the following publications, each of which is incorporated by reference herein in its entirety: U.S. Patent No. 5,521,073 issued May 28, 1996; U.S. Patent No. 5,643,755 issued July 1, 1997; U.S. Patent No. 5,650,490 issued July 22, 1997; U.S. Patent No. 5,814,464 issued September 29, 1998; U.S. Patent No. 5,879,672 issued March 9, 1999; U.S. Patent No. 5,851,797 issued December 22, 1998; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof," published as WO 96/11269 on 18 April 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled "TIE-2 Ligands Methods of Making and Uses Thereof,"published as WO 96/31598 on 10 October 1996 in the name of Regeneron Pharmaceuticals, Inc.; PCT International Application entitled

"TIE-2 Receptor Ligands (TIE Ligand-3; TIE Ligand-4) And Their Uses," published as WO 97/48804 on 24 December 1997 in the name of Regeneron Pharmaceuticals, Inc; and PCT International Application entitled "Modified TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc.

When used herein, fusion polypeptide includes functionally equivalent molecules in which amino acid residues are substituted for residues within the sequence resulting in a silent or conservative change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent or conservative alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc.

Cells that express the fusion polypeptides of the invention are genetically engineered to produce them by, for example, transfection, transduction, electroporation, or microinjection.

The present invention encompasses the nucleic acid sequences encoding the fusion polypeptides of the invention, as well as sequences that hybridize under stringent conditions to nucleic acid sequences that are complementary to the nucleic acid sequences of the invention. Stringent conditions are set forth in, for example, Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). In addition, the present invention encompasses nucleic acid sequences that are different from the nucleic acid sequences of the invention but which nevertheless encode the fusion polypeptides of the invention due to the degeneracy of the genetic code.

In addition, the present invention contemplates use of the fusion polypeptides described herein in tagged forms.

Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion polypeptides of the invention using appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of nucleic acid sequence encoding the fusion polypeptides of the invention may be regulated by a second nucleic acid sequence so that the fusion polypeptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the fusion polypeptides described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the fusion polypeptide include, but are not limited to the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980,

Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the b-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene

control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising Eph fusion polypeptide encoding or angiopoietin fusion polypeptide encoding nucleic acids as described herein, are used to transfect the host and thereby direct expression of such nucleic acid to produce fusion polypeptides which may then be recovered in biologically active form. As used herein, a biologically active form includes a form capable of binding to the relevant receptor and causing a differentiated function and/or influencing the phenotype of the cell expressing the receptor. Such biologically active forms would, for example, induce phosphorylation of the tyrosine kinase domain of the Ehk-1, Elk, or Tie2 receptor, or stimulation of synthesis of cellular DNA.

Expression vectors containing the nucleic acid inserts can be identified by three general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign nucleic acids inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted nucleic acid sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign nucleic acid sequences in the vector. For example, if an efl nucleic acid sequence is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign nucleic acid product expressed by the recombinant. Such assays can be based, for example, on the physical or

functional properties of the nucleic acid product of interest, for example, by binding of a ligand to a receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or binding to antibodies produced against the protein of interest or a portion thereof.

Cells of the present invention may transiently or, preferably, constitutively and permanently express the ephrin or angiopoietin fusion polypeptide as described herein.

The ephrin fusion polypeptides of the invention may be useful in methods of treating a patient suffering from a neurological disorder comprising treating the patient with an effective amount of the ephrin fusion polypeptide.

For example, the Elk receptor is expressed primarily in brain. Accordingly, it is believed that an Elk binding ephrin fusion polypeptide ligand will support the induction of a differential function and/or influence the phenotype, such as growth and/or survival of neural cells that express this receptor.

The present invention also provides for pharmaceutical compositions comprising the ephrin fusion polypeptide in a suitable pharmacologic carrier. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

As our understanding of neurodegenerative disease/neurotrauma becomes clearer, it may become apparent that it would be beneficial to decrease the effect of endogenous Efl-6. Therefore, in areas of nervous system trauma, it may be desirable to provide Efl-6 antagonists, including, but not limited to,

fusion polypeptide forms of Efl-6 which may compete with cell-bound ligand for interaction with Elk receptor. It may be desirable to provide such antagonists locally at the injury site rather than systemically. Use of an Efl-6 antagonist providing implant may be desirable.

Alternatively, certain conditions may benefit from an increase in Efl-6 responsiveness. It may therefore be beneficial to increase the number or binding affinity of Efl-6 in patients suffering from such conditions.

The invention herein further provides for the development of a fusion polypeptide, as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE-2 receptor. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

Because TIE-2 receptor has been identified in association with endothelial cells and, as was previously demonstrated, blocking of agonists of the receptor such as TIE-2 ligand 1 (Ang-1) has been shown to prevent vascularization, applicants expect that TIE-2 agonist fusion polypeptides of the invention may be useful for the induction of vascularization in diseases or disorders where such vascularization is indicated. Such diseases or disorders would include wound healing, ischemia and diabetes. The ligands may be tested in animal models and used therapeutically as described for other agents, such as vascular endothelial growth factor (VEGF), another endothelial cell-specific factor that is angiogenic. Ferrara, et al. U.S. Patent No. 5,332,671 issued July 26, 1994. The Ferrara reference, as well as other studies, describe in vitro and in vivo studies that may be used to demonstrate the effect of an angiogenic factor in enhancing blood flow to ischemic myocardium, enhancing wound healing, and in other therapeutic settings wherein neoangiogenesis is desired. [see Sudo, et al., European Patent Application 0 550 296 A2 published July 7, 1993; Banai, et al. Circulation 89:2183-2189 (1994); Unger, et al. Am. J. Physiol. 266:H1588-H1595

(1994); Lazarous, et al. Circulation 91:145-153 (1995)]. According to the invention, the agonist fusion polypeptides may be used alone or in combination with one or more additional pharmaceutically active compounds such as, for example, VEGF or basic fibroblast growth factor (bFGF).

Conversely, antagonists of the TIE-2 receptor, such as TIE-2 receptorbodies or TIE-2 ligand 2 (Ang-2) as described in Example 9 in International Publication No. WO 96/31598 published 10 October 1996, have been shown to prevent or attenuate vascularization, and are thus expected to be useful in preventing or attenuating, for example, tumor growth. Similarly then, TIE-2 antagonist fusion polypeptides of the invention would also be useful for those purposes. These antagonists may be used alone or in combination with other compositions, such as anti-VEGF antibodies, that have been shown to be useful in treating conditions in which the therapeutic intent is to block angiogenesis.

For example, applicants have determined that TIE-2 ligands are expressed in cells within, or closely associated with, tumors. For example, TIE-2 ligand 2 (Ang-2) appears to be tightly associated with tumor endothelial cells. Accordingly, TIE-2 antagonist fusion polypeptides of the invention may also be useful in preventing or attenuating, for example, tumor growth.

In other embodiments, the TIE-2 agonist fusion polypeptides of the invention described herein may be used as hematopoietic factors. A variety of hematopoietic factors and their receptors are involved in the proliferation and/or differentiation and/or migration of the various cells types contained within blood. Because the TIE-2 receptors are expressed in early hematopoietic cells, the TIE-2 ligands are expected to play a comparable role in the proliferation or differentiation or migration of these cells. Thus, for example, TIE-2 agonist fusion polypeptide compositions may be prepared, assayed, examined in in vitro and in vivo biological systems and

used therapeutically as described in any of the following: Sousa, U.S. Patent No. 4,810,643, Lee, et al., Proc. Natl. Acad. Sci. USA 82:4360-4364 (1985) Wong, et al. Science, 228:810-814 (1985); Yokota, et al. Proc. Natl. Acad. Sci (USA) 81:1070 (1984); Bosselman, et al. WO 9105795 published May 2, 1991 entitled "Stem Cell Factor" and Kirkness, et al. WO 95/19985 published July 27, 1995 entitled "Haemopoietic Maturation Factor". Accordingly, the fusion polypeptides may be used to diagnose or treat conditions in which normal hematopoiesis is suppressed, including, but not limited to anemia, thrombocytopenia, leukopenia and granulocytopenia. In a preferred embodiment, the fusion polypeptides may be used to stimulate differentiation of blood cell precursors in situations where a patient has a disease, such as acquired immune deficiency syndrome (AIDS) which has caused a reduction in normal blood cell levels, or in clinical settings in which enhancement of hematopoietic populations is desired, such as in conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment or chemotherapy.

The fusion polypeptides of the present invention may be used alone, or in combination with another pharmaceutically active agents such as, for example, cytokines, neurotrophins, interleukins, etc. In a preferred embodiment, the fusion polypeptides may be used in conjunction with any of a number of factors which are known to induce stem cell or other hematopoietic precursor proliferation, or factors acting on later cells in the hematopoietic pathway, including, but not limited to, hemopoietic maturation factor, thrombopoietin, stem cell factor, erythropoietin, G-CSF, GM-CSF, etc.

In an alternative embodiment, TIE-2 receptor antagonist fusion polypeptides are used to diagnose or treat patients in which the desired result is inhibition of a hematopoietic pathway, such as for the treatment of myeloproliferative or other proliferative disorders of blood forming organs

such as thrombocythemias, polycythemias and leukemias. In such embodiments, treatment may comprise use of a therapeutically effective amount of the fusion polypeptides as described herein.

Effective doses useful for treating these or other diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975)]. Pharmaceutical compositions for use according to the invention include the fusion polypeptides described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation prior to administration *in vivo*. For example, the pharmaceutical composition may comprise a fusion polypeptide in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

The present invention also provides for pharmaceutical compositions comprising the fusion polypeptides described herein, in a pharmacologically acceptable vehicle. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

Angiopoietin ligands:

As described *supra*, experiments with mutants of Ang-1 and Ang-2 have demonstrated that the fibrinogen domains (FD) are the receptor-binding domains, and that dimerized versions (dimerization occurs due to the interaction between the Fc components of adjacent molecules), for example Ang-1-FD-Fc, can bind to the Tie-2 receptor with much higher affinity than monomeric Ang-1-FD. However, Ang-1-FD-Fc is not able to induce phosphorylation (activate) the Tie-2 receptor on endothelial cells unless it is further clustered with goat anti-human Fc antibodies (Jackson Immunoresearch). For this reason, mutant versions of Ang-1-FD and Ang-2-FD were designed that were intrinsically more highly clustered.

Two general types of nucleic acid molecules were constructed. The first type consisted of two tandem copies of Ang-1-FD fused to an Fc tag, thus leading

to a secreted polypeptide molecule that is dimeric with respect to the Fc tag but tetrameric with respect to Ang-1-FD. Similarly, two tandem copies of Ang-2-FD fused to an Fc tag constituted the angiopoietin-2 version of this type of construct. These molecules were designated Ang-1-FD-FD-Fc and Ang-2-FD-FD-Fc, respectively.

In the second type of nucleic acid molecule constructed, two copies of Ang-1-FD were connected by an Fc tag bridging between them, thus creating the structure Ang-1-FD-Fc-FD that is still dimeric with respect to the Fc, as well as tetrameric with respect to Ang-1-FD. An angiopoietin-2 version was also constructed and these two molecules were designated Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD, respectively.

For either type of construct, similar properties were observed: unlike dimeric Ang-1-FD-Fc, which fails to activate Tie-2 in endothelial cells, both Ang-1-FD-FD-Fc and Ang-1-FD-Fc-FD could readily activate Tie-2 in endothelial cells, with a potency comparable to that of the native ligand. Also, like native angiopoietin-2, Ang-2-FD-Fc-FD could antagonize angiopoietin-1 activity with a potency that is comparable to that of native angiopoietin-2, and with much greater potency than the marginally antagonistic activity of the Ang-2-FD-Fc dimer.

Construction of mutant angiopoietin nucleic acid molecules.

All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into the mammalian expression vector pMT21 (Genetics

Institute, Inc.) with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5′ end to promote protein translation. The bridging sequences described *infra* were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

Example 1: Construction of the Ang-1-FD-FD-Fc, Ang-2-FD-FD-Fc, Ang-1-FD-Fc-FD, and Ang-2-FD-Fc-FD nucleic acid molecules.

Ang-1-FD-FD-Fc: Ang-1-FD-FD-Fc consists of a trypsin signal sequence at its amino terminus to allow for secretion (bases 1-45 of Figure 1A) followed by the angiopoietin-1 fibrinogen domain (FD) (bases 46-690 of Figure 1A-Figure 1B), a short bridging sequence consisting of the amino acids Gly-Pro Ala-Pro (bases 691-702 of Figure 1B), a second angiopoietin-1 FD (bases 703-1750 of Figure 1B-Figure 1D), another bridging sequence consisting of the amino acids Gly-Pro-Gly (bases 1351-1359 of Figure 1D), and the coding sequence for the Fc portion of human IgG1 (bases 1360-2058 of Figure 1D-Figure 1E).

Ang-2-FD-FD-Fc: The Ang-2-FD-FD-Fc nucleic acid molecule was similarly constructed. It consists of a trypsin signal sequence (bases 1-45 of Figure 2A), an angiopoietin-2 FD (bases 46-690 of Figure 2A- Figure 2B), a bridging amino acid sequence Gly-Gly-Pro-Ala-Pro (bases 691-705 of Figure 2B), a second angiopoietin-2 FD (bases 706-1353 of Figure 2B-Figure 2D), another bridging amino acid sequence Gly-Pro-Gly (bases 1354-1362 of Figure 2D), and the coding sequence for the Fc portion of human IgG1 (bases 1363-2061 of Figure 2D-Figure 2E.

Ang-1-FD-Fc-FD: The Ang-1-FD-Fc-FD consists of a trypsin signal sequence (bases 1-45 of Figure 3A), an angiopoietin-1 FD (bases 46-690 of Figure 3A-3B), the bridging amino acid sequence Gly-Pro-Gly (bases 691-699 of Figure

3B), the coding sequence for the Fc portion of human IgG1 (bases 700-1395 of Figure 3B-3D), another bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1396-1419 of Figure 3D), and a second angiopoietin-1 FD (bases 1420-2067 of Figure 3D-Figure 3E).

Ang-2-FD-Fc-FD: The Ang-2-FD-Fc-FD nucleic acid molecule consists of a trypsin signal sequence (bases 1-45 of Figure 4A), an angiopoietin-2 FD domain (bases 46-690 of Figure 4A-Figure 4B), the bridging amino acid sequence Gly-Gly-Pro-Gly (bases 691-702 of Figure 4B), the coding sequence for the Fc portion of human IgG1 (bases 703-1398 of Figure 4B- Figure 4D), the bridging amino acid sequence Gly-Gly-Gly-Gly-Ser-Gly-Ala-Pro (bases 1399-1422 of Figure 4D), and a second angiopoietin-2 FD (bases 1423-2067 of Figure 4D-Figure 4E).

Example 2: Characterization of Ang-1 FD-Fc-FD protein.

Molecular Weight Analysis: The predicted molecular weight for Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses of COS cell-derived protein described *infra* confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions (Figure 5). Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To

determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. Figure 6 shows the results of this analysis using COS cell-derived protein. The molecular weight of the dimeric protein appears to be approximately 200kD and presence of a single peak implies that the protein solution is, in fact, homogenous.

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang1-FD-Fc-FD DNA construct described *supra*. All transfections were performed using standard techniques known in the art. The COS cell supernatant was analyzed using Biacore technology (Pharmacia, Inc.) to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 765, which is equivalent to 0.9mg of recombinant protein/liter of COS cell supernatant. These values represent very high levels of expression.

Purification of COS Supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). In fact, the relative ease of purification of the Ang-1-FD-Fc-FD protein gives it a distinct advantage over the parent protein, angiopoietin-1, from which

it is derived, and the mutant version of angiopoietin-1 called Ang1* that consists of the N-terminal of angiopoietin-2 fused to the coiled-coil domain and fibrinogen domain of angiopoietin-1 and that has a Cys to Ser mutation at amino acid 245. (See PCT International Application entitled "Modified TIE-2 Receptor Ligands," published as WO 98/05779 on 12 February 1998 in the name of Regeneron Pharmaceuticals, Inc., especially Figure 27, which is hereby incorporated by reference).

Both angiopoietin-1 and Ang1* require extensive, expensive and labor-intensive purification schemes that result in relatively poor yields of recombinant protein. The need for cost-effective, simple purification schemes for biologicals intended for clinical use can not be over-emphasized.

The COS cell supernatant was purified as described *supra* and yielded approximately 1 mg of purified Ang-1-FD-Fc-FD protein that was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of COS cell-derived Ang-1-FD-Fc-FD protein: Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. This was of concern because the mutant molecule, Ang1*, has a history of containing between 10-20% N-terminally truncated species. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

Receptor binding analysis of COS cell-derived Ang-1-FD-Fc-FD: Previous studies have determined that the fibrinogen domain (FD) of the angiopoietin molecules is necessary for ligand/receptor interaction. Furthermore, in order for high affinity binding to the Tie-2 receptor to

occur, native angiopoietin-1, angiopoietin-2, and the mutant Ang1* must form at least tetrameric, and possibly higher order, multimers. To determine whether the COS cell-derived Ang-1-FD-Fc-FD protein, which is tetrameric with respect to the FD domain, could bind to Tie-2 with high affinity, standard Biacore analysis was performed. Briefly, Tie-2-Fc receptor body protein, which is a fusion protein comprising the ectodomain of Tie-2 fused to the Fc domain of human IgG1, was immobilized on a Biacore chip. Ang-1-FD-Fc-FD-containing solution was passed over the chip and binding between Tie-2 ectodomain and Ang-1-FD-Fc-FD was allowed to occur. The binding step was followed by a 0.5 M NaCl high salt wash. The high salt wash was not able to disrupt the interaction between the Ang-1-FD-Fc-FD protein and the Tie-2 receptor ectodomain, implying that there is a strong interaction between the mutant ligand and receptor. This result is consistent with earlier Biacore results in which both Ang-1-FD-Fc-FD parent molecule, angiopoietin-1 and the mutant Ang1* molecule, have been shown to interact strongly with the Tie-2-Fc receptor and that this interaction is not disrupted by high salt. In contrast, several mutant molecules derived from the parent angiopoietin-1 molecule are readily dissociated from the Tie-2-Fc receptor when treated with high salt. The mutant molecules, designated Ang-1/FD (a monomer with respect to the FD), Ang-1/FD-Fc (also a monomer with respect to the FD, but which is able to form a dimer due to the presence of the Fc domain), and Ang-1/C/FD (a monomer with respect to the FD, but which also contains the coiled-coil domain of angiopoietin-1), do not exist in multimeric forms sufficient for high affinity binding to the Tie-2 receptor.

Example 3: Characterization of COS cell-derived Ang-2-FD-Fc-FD protein.

Molecular Weight Analysis: As described for Ang-1-FD-Fc-FD *supra*, the predicted molecular weight for Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form of Ang-2-FD-Fc-FD has a predicted

weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104. SDS PAGE analyses of COS cell-derived protein confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 88kD under reducing conditions (Figure 7). Light scatter analysis confirmed the molecular weight (171kD) and revealed that the Ang-2-FD-Fc-FD protein, like Ang-1-FD-Fc-FD, exists as a homogeneous species (Figure 8).

Expression Level in COS Cells: COS cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by transiently transfecting COS cells with the Ang-2-FD-Fc-FD DNA construct described *supra*. The COS cell supernatant was analyzed by Biacore to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis resulted in an RU value of 606, which is equivalent to 0.7mg of recombinant protein/liter of COS cell supernatant. These values represent relatively high levels of expression.

Purification of COS Supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The COS cell supernatant was purified as described for Ang-1-FD-Fc-FD supra and yielded approximately 2 mg of purified Ang-2-FD-Fc-FD protein that was used in the studies described *infra* to further characterize this protein.

N-terminal sequencing: Purified COS cell-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Arg-Asp-X-Ala-Glu, wherein X is Cys. This

sequence can be found at amino acids 16-20 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Receptor binding analysis of COS cell-derived protein: To determine whether the COS cell-derived Ang-2-FD-Fc-FD protein could bind to the Tie-2 receptor, standard Biacore analysis was performed as described for Ang-1-FD-Fc-FD *supra*. As with Ang-1-FD-Fc-FD, a high salt wash was not able to disrupt the interaction between the Ang-2-FD-Fc-FD protein and the Tie-2-Fc receptor, again implying that there is a strong interaction between mutant ligand and receptor.

Example 4: Effects of COS cell-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

Because Ang-1-FD-Fc-FD is a mutant molecule derived from the agonist angiopoietin-1 and Ang-2-FD-Fc-FD is a mutant molecule derived from the antagonist angiopoietin-2, we wanted to determine whether or not these two mutant molecules would retain the activity associated with the parent molecule from which it was derived.

Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA 80:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

(A) Ang1*-mediated vs. Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with either 0.1 μg/ml, 0.2 μg/ml, or 0.8 μg/ml Ang1* or Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 9).

(B) Ability of Ang-2-FD-Fc-FD to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.4 μg/ml of the Tie-2 agonist Ang1* and 1 μg/ml, 2 μg/ml, 4 μg/ml. 6 μg/ ml, or 8 μg/ml of Ang-2-FD-Fc-FD. As shown in Figure 10, Ang-2-FD-Fc-FD is able to block Ang1* stimulation of the Tie-2 receptor when it is present in at least a 10-15 fold molar excess of Ang1*.

(C) Ability of angiopoietin-2 to block Ang1*-mediated Tie-2 receptor phosphorylation in EAhy926 cells: To compare the blocking effects of the naturally occurring antagonist angiopoietin-2 with that of Ang-2-FD-Fc-FD, the same experiment described in (B) *supra* was performed, substituting angiopoietin-2 for Ang-2-FD-Fc-FD. The results of this experiment are shown in Figure 11. At a 20 fold molar excess, the angiopoietin-2 has not reduced the phosphorylation level to 50%. This result, coupled with the results described in (B) *supra* implies that Ang-2-FD-Fc-FD is a more potent inhibitor or Ang1*-mediated Tie-2 receptor phosphorylation than angiopoietin-2.

(D) Ability of Ang-2-FD-Fc-FD to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the naturally occurring Tie-2 agonist angiopoietin-1 and 1 μg/ml, 2 μg/ml, 4 μg/ml. 6 μg/ ml, or 8 μg/ml of Ang-2-FD-Fc-FD. The results of this experiment, shown in Figure 12, show that while there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, Ang-2-FD-Fc-FD seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 10 and described in (B) *supra*.

(E) Ability of angiopoietin-2 to block angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the angiopoietin-1 and 1 μg/ml, 2 μg/ml, 4 μg/ml,

6 μg/ ml, or 8 μg/ml of angiopoietin-2. The results of this experiment, shown in Figure 13, show that there is a trend toward blocking angiopoietin-1-mediated phosphorylation of the Tie-2 receptor in these cells, but, like Ang-2-FD-Fc-FD, angiopoietin-2 seems to be more effective at blocking Ang1*-mediated phosphorylation of Tie-2, as shown in Figure 11 and described in (C) *supra*.

Example 5: Construction of Ang-1-FD-Fc-FD CHO cell expression vector pRG763/Ang-1-FD-Fc-FD.

The pRG763/Ang-1-FD-Fc-FD CHO cell expression vector was constructed by isolating from the plasmid pCDNA3.1/Ang1-FD-Fc-FD a 2115 base pair EcoRI - NotI fragment containing Ang1-FD-Fc-FD and ligating this fragment into pRG763 vector digested with EcoRI and NotI. A large scale (2L) culture of E. coli DH10B cells carrying the pRG763/Ang-1-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was verified by digestion of aliquots with NcoI and HincII restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

Example 6: Expression of Ang-1-FD-Fc-FD in CHO cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μg of pRG763/Ang-1-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10%

FBS was added. Plates were incubated at 37°C in a 5% CO_2 incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *infra*.

Example 7: Construction of Ang-2-FD-Fc-FD CHO cell expression vector pRG763/Ang-2-FD-Fc-FD.

The plasmid pRG763/Ang-2-FD-Fc-FD was constructed by isolating from the plasmid pCDNA3.1/Ang-2-FD-Fc-FD a 2097 base pair EcoRI - NotI fragment containing Ang-2-FD-Fc-FD and ligating this fragment into the pRG763 vector digested with EcoRI and NotI. A large scale (1L) culture of E. coli DH10B cells carrying the pRG763/Ang-2-FD-Fc-FD plasmid was grown overnight in TB + ampicillin and the plasmid DNA was extracted using a Promega Wizard Plus Maxiprep kit, following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of plasmid DNA with NcoI and Ppu10I restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

Example 8: Expression of Ang-2-FD-Fc-FD in CHO cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 μg of pRG763/Ang-2-FD-Fc-FD using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after

adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO_2 incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles purified as described infra.

Example 9: Characterization of stable CHO clone-derived Ang-1-FD-Fc-FD protein.

Molecular Weight Analysis: The predicted molecular weight for stable CHO clone-derived Ang-1-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT) The monomeric form (with respect to the Fc) has a predicted weight of 76,349. In addition, there are three predicted N-linked glycosylation sites, approximately 2500 MW/site, that could potentially increase the molecular weight of the monomeric protein to 83,849. Due to the interaction between the Fc components of adjacent molecules, the protein actually exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,698. Subsequent SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 210kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis was performed to further confirm the molecular weight and, more importantly, determine whether or not the protein was a homogeneous species. Light scattering is a function of mass and concentration of a macromolecule. To determine molecular weight, the protein sample was injected onto a gel filtration column and the effluent was monitored with an on line light scattering detector and a refractive index and/or a UV detector. The light scattering detector is a MiniDawn laser light scattering detector was from Wyatt Technology

Corporation (Santa Barbara, CA). This instrument measures static light at three different angles. The on line refractive index detector or UV detector serve to measure protein concentration. Astra 4.7 Software (Wyatt Technology Corporation, Santa Barbara, CA) was used to calculate the protein concentration based on either dn/dc (dn = change of refractive index; dc = concentration) or the extinction coefficient of the protein. The molecular weight of protein is then calculated from the angular dependence of light scattering. The results of this analysis show that the dimeric protein appears to be approximately 173.9kD and the presence of a single peak implies that the protein solution is homogenous.

Expression level of Ang-1-FD-Fc-FD in stable CHO clones: CHO cell supernatant containing recombinant Ang-1-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-1-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-1-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of 2-3 pg/cell/day.

Purification of Ang-1-FD-Fc-FD protein derived from stable CHO clone supernatants: Because the Ang-1-FD-Fc-FD protein contains an Fc domain, purification is relatively simple and straight forward using standard Protein A column chromatography (Pharmacia, Inc.) followed by standard size exclusion chromatography (Pharmacia, Inc.). The CHO cell supernatant was purified as described *supra* and the purified ANG-1-FD-Fc-FD protein was used in the studies described *infra* to further characterize the protein.

N-terminal sequencing of stable CHO clone-derived Ang-1-FD-Fc-FD protein: Purified Ang-1-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal

sequence, Arg-Asp-X-Ala-Asp, wherein X is Cys. This sequence can be found at amino acids 16-20 of Figure 3A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 Figure 3A.

Example 10: Characterization of stable CHO clone-derived Ang-2-FD-Fc-FD protein.

Molecular Weight Analysis: As described for stable CHO clone-derived Ang-1-FD-Fc-FD *supra*, the predicted molecular weight for stable CHO clone-derived Ang-2-FD-Fc-FD protein was determined using the MacVector Program (Kodak, Scientific Imaging Systems, New Haven, CT). The monomeric form of Ang-2-FD-Fc-FD has a predicted weight of 76,052, with three predicted N-linked glycosylation sites that could potentially increase the molecular weight of the monomeric protein to 83,552. Like Ang-1-FD-Fc-FD, the protein exists as a dimer with a predicted molecular weight, including possible N-linked glycosylation, of 167,104. SDS PAGE analyses confirmed these approximate molecular weights, with a band running at about 200kD under non-reducing conditions and a band running at about 85kD under reducing conditions. Light scatter analysis confirmed the molecular weight (176.6kD) and revealed that the stable CHO clone-derived Ang-2-FD-Fc-FD protein, like stable CHO clone-derived Ang-1-FD-Fc-FD, exists as a homogeneous species.

Expression level of Ang-2-FD-Fc-FD derived from stable CHO clones: CHO cell supernatant containing recombinant Ang-2-FD-Fc-FD protein was generated by stably transfecting CHO cells with the Ang-2-FD-Fc-FD DNA construct described *supra*. The CHO cell supernatant was analyzed by standard ELISA using an anti-human IgG antibody as a capture antibody and an anti-human IgG antibody conjugated to alkaline phosphatase as a reporter antibody to quantitate the amount of Ang-2-FD-Fc-FD protein present in the supernatant. This analysis revealed expression levels of approximately 1-2 pg/cell/day.

Purification of stable CHO clone-derived Ang-2-FD-Fc-FD from cell supernatants: As with Ang-1-FD-Fc-FD, Ang-2-FD-Fc-FD protein contains an Fc domain, so purification is relatively simple and straight forward using standard Protein A column chromatography followed by standard size exclusion chromatography. The CHO cell supernatant was purified as described for stable CHO clone-derived Ang-1-FD-Fc-FD *supra* and was used in the studies described *infra* to further characterize this protein.

N-terminal sequencing of stable CHO clone-derived Ang-2-FD-Fc-FD protein: Purified stable CHO clone-derived Ang-2-FD-Fc-FD protein was subjected to standard N-terminal sequence analysis to determine if any truncated species of the protein were being generated. The analysis revealed only one N-terminal sequence, Asp-X-Ala-Glu-Val, wherein X is Cys. This sequence can be found at amino acids 17-21 of Figure 4A, and immediately follows the protein's signal sequence corresponding to amino acids 1-15 of Figure 4A.

Example 11: Effects of stable CHO clone-derived Ang-1-FD-Fc-FD and Ang-2-FD-Fc-FD on Tie-2 receptor phosphorylation in EAhy926 cells.

Assay system: All of the experiments described *infra* utilized the cell line EAhy926 (Edgell, C. J., et al., (1983) Proc. Natl. Acad. Sci. USA 80:3734-3737) and standard phosphorylation assays and reagents familiar to those of skill in the art.

(A) Ang1*-mediated vs. stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were stimulated with 0.4 μg/ml Ang1* or 0.2 μg/ml or 0.4 μg/ml stable CHO clone-derived Ang-1-FD-Fc-FD protein. A standard phosphorylation assay revealed that or stable CHO clone-derived Ang-1-FD-Fc-FD was equivalent to Ang1* in its ability to stimulate phosphorylation of the Tie-2 receptor in EAhy926 cells (Figure 17).

(B) Ability of stable CHO clone-derived Ang-2-FD-Fc-FD to block stable CHO clone-derived Ang-1-FD-Fc-FD-mediated Tie-2 receptor phosphorylation in EAhy926 cells: EAhy926 cells were treated with 0.2 μg/ml of the Tie-2 agonist Ang-1-FD-Fc-FD and 2 μg/ml, 4 μg/ml, 8 μg/ml or 16 μg/ml of stable CHO clone-derived Ang-2-FD-Fc-FD. As shown in Figure 18, Ang-2-FD-Fc-FD is able to block stable CHO clone-derived Ang-1-FD-Fc-FD stimulation of the Tie-2 receptor when it is present in at least a 40 fold molar excess of stable CHO clone-derived Ang-1-FD-Fc-FD.

Ephrin ligands:

In previous experiments (Davis et al., 1994, Science, 266:816-819; Gale et al., 1996, Neuron 17:9-19, Gale and Yancopoulos, 1997, Cell Tissue Research 290:227-241), soluble, unclustered Ephrin-B1-Fc and Ephrin-B2-Fc, which dimerize at their respective Fc domains and therefore are dimeric with respect to either the Ephrin-B1 or Ephrin-B2 ectodomain, failed to induce EphB2 receptor phosphorylation. However, when either molecule was multimerized by pre-clustering with an anti-Fc antibody, they became potent agonists for the EphB2 receptor, as judged by tyrosine phosphorylation of the EphB2 receptor in a COS cell reporter assay. Because multimerization of both Ephrin-B1 and Ephrin-B2 appears to be necessary for induction of receptor phosphorylation, we theorized that a molecule that contained tandem repeats of either Ephrin-B1 or Ephrin-B2 ectodomains fused to an Fc domain, which would be dimeric with respect to the Fc domain but which would be tetrameric with respect to Ephrin ectodomains, might be sufficiently clustered to induce receptor phosphorylation. To test this hypothesis, the following DNA constructs were constructed, recombinant proteins produced, and reporter assays performed.

Construction of tandem Ephrin ectodomain/Fc domain nucleic acid molecules.

All of the following nucleic acid molecules were constructed by standard recombinant DNA techniques (See e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY), sequence-verified by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA), and subcloned into either the mammalian expression pJFE14 (Ephrin-B1-Ephrin-B1-Fc) or pMT21 (Ephrin-B2-Ephrin-B2-Fc), each with a Kozak sequence (Kozak, M., 1987, Nucleic Acids Res. 15:8125-8148) at the 5′ end to promote protein translation. The bridging sequences described *infra* were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains, but there is no indication that there is a very critical nature to these bridging sequences (though varying the length of the linker in some of these constructs led to some variation in the amount of protein produced).

Example 12: Construction of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules.

(A) Ephrin-B1-Ephrin-B1-Fc: The Ephrin-B1-Ephrin-B1-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B1 (Davis et al., ibid.), which corresponds to nucleotides 1-711 of Figure 14A-Figure 14B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 712-720 of Figure 14B), followed by a second copy of the ectodomain of Ephrin-B1 (corresponding to nucleotides 721-1344 of Figure 14B-Figure 14D), except that in this copy of the Ephrin-B1 ectodomain the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1345-1353 of Figure 14D),

followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1354-2049 of Figure 14D-Figure 14E).

(B) Ephrin-B2-Ephrin-B2-Fc: The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E).

As with the angiopoietin nucleic acid molecules described *supra*, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

Example 13: Expression of tandem Ephrin recombinant proteins in COS cells.

COS cells were transiently transfected with either the Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc nucleic acid molecules described *supra* using standard transfection techniques known in the art. Two days subsequent to transfection, the growth medium (DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 10% calf serum) was aspirated and replaced with serum-free medium (DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine). Cell were grown for an additional three days and then the serum-free medium containing the recombinant proteins was collected. Recombinant protein concentration was determined by performing dot blots and comparing the

signal obtained to a standard curve. Once approximate protein concentrations were determined, the Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc recombinant proteins were used in the cell reporter assays described *infra*.

Example 14: Characterization of the COS cell-derived tandem Ephrin ectodomain/Fc domain recombinant proteins.

Reporter Assay: COS cells, which endogenously express the Eph family receptor EphB2 (Gale et al., 1996, Neuron 17:9-19), were used in reporter assays to evaluate the ability of Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc to induce receptor phosphorylation. The assays were performed as previously described (Davis et al., ibid.; Gale et al., ibid.). Briefly, COS cells were grown to 80-90% confluency in standard growth medium described *supra*. After growth, the medium was aspirated, and replaced with serum-free medium (described *supra*) for 1-2 hours prior to treatment with either Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc recombinant protein. The cells were stimulated with 500 ng/ml Ephrin-B1-Ephrin-B1-Fc or Ephrin-B2-Ephrin-B2-Fc for 30 minutes at 37°C, with or without affinity purified human IgG1 Fc-specific goat anti-human antibody (Jackson Immunoresearch, West Grove, PA) at a final concentration of 17 µg/ml. This antibody is capable of clustering the Fc tagged fusion. Subsequent to treatment, the COS cells were harvested and cell lysates were prepared as described in Davis, et al. and Gale, et al., *supra*. The EphB2 receptor protein was immunoprecipitated from the cell lysates using an anti-EphB2 antisera (Henkemeyer et al., 1994, Oncogene 9:1001-1014). Immunoprecipitates were resolved by standard SDS PAGE and transferred to PVDF membranes (Millipore) for western blot analysis. The membranes were probed with either anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology Institute, Lake Placid, NY) or anti-EphB2 antibodies (Henkemeyer, et al., ibid.) to determine the extent of EphB2

phosphorylation and the relative quantities of EphB2 in the experimental conditions described *supra*.

Results: Both Ephrin-B1-Ephrin-B1-Fc and Ephrin-B2-Ephrin-B2-Fc were shown to be approximately as active as anti-Fc antibody-clustered Ephrin-B1-Fc in their ability to induce EphB2 receptor phosphorylation in the COS cell reporter assay. Furthermore, if either of the proteins were further clustered with the goat anti-human Fc antibody, they became even more potent in their ability to induce EphB2 receptor phosphorylation. Figure 16 shows the results of this phosphorylation assay.

Example 15: Construction of Ephrin-B2-Ephrin-B2-Fc CHO expression vector.

The Ephrin-B2-Ephrin-B2-Fc DNA molecule consists of the coding sequence of the ectodomain of Ephrin-B2 (Bergemann et al., 1995, Mol. Cell Biol. 15:4821-4929), which corresponds to nucleotides 1-675 of Figure 15A-Figure 15B, followed by a bridging sequence consisting of the amino acids Gly-Pro-Gly (nucleotides 676-684 of Figure 15B), followed by a second copy of the ectodomain of Ephrin-B2 (corresponding to nucleotides 685-1270 of Figure 15B-Figure 15D), except that in this copy the signal sequence has been removed. This second copy is followed by a second Gly-Pro-Gly amino acid bridge (nucleotides 1270-1278 of Figure 15D), followed by the coding sequence for the Fc portion of human IgG1 (nucleotides 1279-1977 of Figure 15D-Figure 15E). This molecule was subcloned into the HindIII and NotI polylinker sites in the expression vector pRG763 and was designated pRG763-m(Ephrin-B2)2-Fc. As with the angiopoietin nucleic acid molecules described *supra*, the bridging sequences were introduced to provide convenient restriction sites and to give flexibility to the junctions between the domains.

Example 16: Expression of Ephrin-B2-Ephrin-B2-Fc in CHO-K1 (E1A) cells.

Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 µg of pRG763-m(Ephrin-B2)2-Fc using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO_2 incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days. After 3 days of incubation the media was aspirated from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and purified as described *supra*.

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises a first subunit comprising at least one copy of the receptor binding domain of a ligand, the first subunit being fused to the N-terminal end of a multimerizing component, said multimerizing component being fused at its C-terminal end to a second subunit comprising at least one copy of the receptor binding domain of a ligand.

2. The nucleic acid of claim 1, wherein the receptor binding domains of the first and second subunits are copies of the receptor binding domain of the same ligand.

3. The nucleic acid of claim 1, wherein the receptor binding domains of the first subunit are copies of the receptor binding domain of a different ligand from the receptor binding domains of the second subunit.

4. The nucleic acid of claim 2, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.

5. The nucleic acid of claim 3, wherein the first and second subunits each have one copy of the receptor binding domain of the ligand.

6. The nucleic acid of claim 2, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

7. The nucleic acid of claim 4, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

8. The nucleic acid of claim 2, wherein the ligand is selected from the group consisting of the EPH family of ligands.

9. The nucleic acid of claim 4, wherein the ligand is selected from the group consisting of the EPH family of ligands.

10. The nucleic acid of claims 1 through 9, wherein the multimerizing component comprises an immunoglobulin derived domain.

11. The nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

12. A fusion polypeptide encoded by the nucleic acid molecule of claims 1 through 11.

13. A composition comprising a multimer of the fusion polypeptide of claim 12.

14. The composition of claim 13, wherein the multimer is a dimer.

15. A vector which comprises the nucleic acid molecule of claims 1 through 11.

16. An expression vector comprising a nucleic acid molecule of claims 1 through 11, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.

18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.

20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.

22. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 17 through 21, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

23. A nucleic acid encoding a fusion polypeptide wherein the fusion polypeptide comprises more than one copy of the receptor binding domain of a ligand in tandem, and wherein either the N-terminal or the C-terminal receptor binding domain is also fused to a multimerizing component.

24. The nucleic acid of claim 23, wherein the receptor binding domains are fused contiguously.

25. The nucleic acid of claim 23, wherein the ligand is not a member of the EPH family of ligands.

26. The nucleic acid of claim 24, wherein the ligand is not a member of

the EPH family of ligands.

27. The nucleic acid of claim 23, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

28. The nucleic acid of claim 24, wherein the receptor binding domain is the fibrinogen domain of angiopoietin-1 or angiopoietin-2.

29. The nucleic acid of claims 23 through 28, wherein the multimerizing component comprises an immunoglobulin derived domain.

30. The nucleic acid molecule of claim 29, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

31. A fusion polypeptide encoded by the nucleic acid molecule of claims 23 through 30.

32. A composition comprising a multimer of the fusion polypeptide of claim 31.

33. The composition of claim 32, wherein the multimer is a dimer.

34. A vector which comprises the nucleic acid molecule of claims 23 through 30.

35. An expression vector comprising a nucleic acid molecule of claims 23 through 30, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

36. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 35, in a suitable host cell.

37. The host-vector system of claim 36, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

38. The host-vector system of claim 36, wherein the suitable host cell is E. coli.

39. The host-vector system of claim 36, wherein the suitable host cell is a COS cell.

40. The host-vector system of claim 36, wherein the suitable host cell is a CHO cell.

41. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claims 36 through 40, under conditions permitting production of the fusion polypeptide and recovering the polypeptide so produced.

Figure 1A

```
          10          20          30          40
     *    *    *    *    *    *    *    *    *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
___a___a___a___a_TRYPSIN SIGNAL SEQUENCE___a___a___a___a___>

     50          60          70          80          90
     *    *    *    *    *    *    *    *    *
AGA GAC TGT GCA GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA
Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          100         110         120         130
     *    *    *    *    *    *    *    *    *
ATC TAC ACT ATT TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG
Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     140         150         160         170         180
     *    *    *    *    *    *    *    *    *
TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA
Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          190         200         210         220
     *    *    *    *    *    *    *    *    *
CAT CGT GAA GAT GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA
His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     230         240         250         260         270
     *    *    *    *    *    *    *    *    *
TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG
Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          280         290         300         310
     *    *    *    *    *    *    *    *    *
AAT GAG TTT ATT TTT GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA
Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     320         330         340         350         360
     *    *    *    *    *    *    *    *    *
AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG
Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          370         380         390         400
     *    *    *    *    *    *    *    *    *
TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG
Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     410         420         430         440         450
     *    *    *    *    *    *    *    *    *
TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG

Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>
```

Figure 1B

```
          460          470          480          490
      *    *    *    *    *    *    *    *    *
ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC
Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   500          510          520          530          540
     *    *    *    *    *    *    *    *    *
AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          550          560          570          580
      *    *    *    *    *    *    *    *    *
TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   590          600          610          620          630
     *    *    *    *    *    *    *    *    *
GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC
Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          640          650          660          670
      *    *    *    *    *    *    *    *    *
TTC AAA GGG CCC AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT
Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   680          690          700          710          720
     *    *    *    *    *    *    *    *    *
CGA CCT TTA GAT TTT GGC CCC GCG CCT TTT AGA GAC TGT GCA GAT
Arg Pro Leu Asp Phe>
___ANG1 FIBRINO____>
                  Gly Pro Ala Pro>
                  ___GPAP BRI____>
                                  Phe Arg Asp Cys Ala Asp>
                                  ___ANG1 FIBRINOGEN-____>

          730          740          750          760
      *    *    *    *    *    *    *    *    *
GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT
Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

   770          780          790          800          810
     *    *    *    *    *    *    *    *    *
ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT
Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

          820          830          840          850
      *    *    *    *    *    *    *    *    *
GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA
Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>
```

Figure 1C

```
860        870         880        890        900
 *     *     *     *     *     *     *     *     *
AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT
Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

      910        920        930        940
 *     *     *     *     *     *     *     *     *
GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

950        960         970        980        990
 *     *     *     *     *     *     *     *     *
GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG
Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

      1000       1010       1020       1030
 *     *     *     *     *     *     *     *     *
GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC
Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

1040       1050        1060       1070       1080
 *     *     *     *     *     *     *     *     *
ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC
Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

      1090       1100       1110       1120
 *     *     *     *     *     *     *     *     *
ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT GCT
Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

1130       1140        1150       1160       1170
 *     *     *     *     *     *     *     *     *
GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA
Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

      1180       1190       1200       1210
 *     *     *     *     *     *     *     *     *
TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC
Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>

1220       1230        1240       1250       1260
 *     *     *     *     *     *     *     *     *
CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT
Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His>
___d___d___d___ANG1 FIBRINOGEN-LIKE DOMAIN_d___d___d___d___>
```

Figure 1D

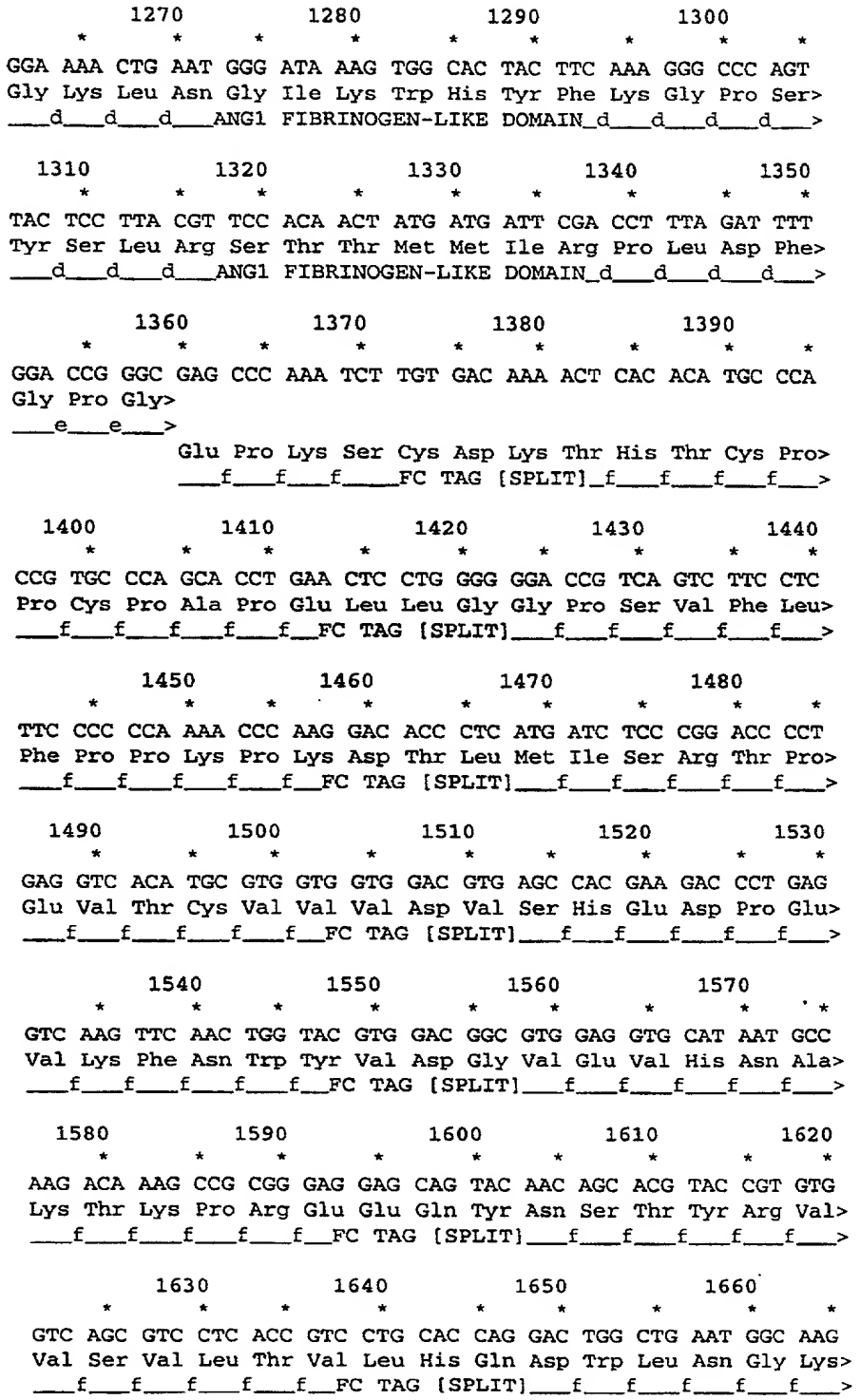


Figure 1E

```
 1670         1680         1690         1700         1710
    *    *    *    *    *    *    *    *    *
GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

         1720         1730         1740         1750
    *    *    *    *    *    *    *    *    *
GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

 1760         1770         1780         1790         1800
    *    *    *    *    *    *    *    *    *
GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

         1810         1820         1830         1840
    *    *    *    *    *    *    *    *    *
GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

 1850         1860         1870         1880         1890
    *    *    *    *    *    *    *    *    *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

         1900         1910         1920         1930
    *    *    *    *    *    *    *    *    *
ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

 1940         1950         1960         1970         1980
    *    *    *    *    *    *    *    *    *
AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

         1990         2000         2010         2020
    *    *    *    *    *    *    *    *    *
TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr>
___f___f___f___f___f__FC TAG [SPLIT]___f___f___f___f___f___>

 2030         2040         2050
    *    *    *    *    *    *
CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
___f___f___f__FC TAG [SPLIT]___f___f___f___>
```

Figure 2A

```
          10          20          30          40
     *    *    *    *    *    *    *    *    *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
___a___a___a___a_TRYPSIN SIGNAL SEQUENCE___a___a___a___a___>

     50          60          70          80          90
     *    *    *    *    *    *    *    *    *
AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC
Arg Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

          100         110         120         130
     *    *    *    *    *    *    *    *    *
ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC
Ile Tyr Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

     140         150         160         170         180
     *    *    *    *    *    *    *    *    *
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG
Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

          190         200         210         220
     *    *    *    *    *    *    *    *    *
CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA
Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

     230         240         250         260         270
     *    *    *    *    *    *    *    *    *
TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA
Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

          280         290         300         310
     *    *    *    *    *    *    *    *    *
AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT
Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

     320         330         340         350         360
     *    *    *    *    *    *    *    *    *
AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG
Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

          370         380         390         400
     *    *    *    *    *    *    *    *    *
TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT
Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

     410         420         430         440         450
     *    *    *    *    *    *    *    *    *
CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC

His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>
```

Figure 2B

```
          460         470         480         490
     *    *    *    *    *    *    *    *    *    *
AGC CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC
Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

   500         510         520         530         540
     *    *    *    *    *    *    *    *    *    *
AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG
Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

          550         560         570         580
     *    *    *    *    *    *    *    *    *    *
TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

   590         600         610         620         630
     *    *    *    *    *    *    *    *    *    *
CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

          640         650         660         670
     *    *    *    *    *    *    *    *    *    *
TGG AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC
Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile>
___b___b___b__ANG2 FIBRINOGEN-LIKE DOMAIN #1___b___b___b___>

   680         690         700         710         720
     *    *    *    *    *    *    *    *    *    *
CGA CCA GCA GAT TTC GGG GGC CCC GCG CCT TTC AGA GAC TGT GCT
Arg Pro Ala Asp Phe>
___ANG2 FIBRINO____>
                    Gly Gly Pro Ala Pro>
                    ___GGPAP BRIDGE____>
                                        Phe Arg Asp Cys Ala>
                                        ___ANG2 FIBRINO____>

          730         740         750         760
     *    *    *    *    *    *    *    *    *    *
GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA
Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

   770         780         790         800         810
     *    *    *    *    *    *    *    *    *    *
ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG
Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

          820         830         840         850
     *    *    *    *    *    *    *    *    *    *
GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT
Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>
```

Figure 2C

```
860          870          880          890          900
 *    *    *    *    *    *    *    *    *
GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

        910          920          930          940
    *    *    *    *    *    *    *    *    *
TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT
Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

   950          960          970          980          990
    *    *    *    *    *    *    *    *    *
TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT
Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

        1000         1010         1020         1030
    *    *    *    *    *    *    *    *    *
AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC
Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

  1040         1050         1060         1070         1080
    *    *    *    *    *    *    *    *    *
TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA
Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

        1090         1100         1110         1120
    *    *    *    *    *    *    *    *    *
CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC CAA CCA GGA
Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

  1130         1140         1150         1160         1170
    *    *    *    *    *    *    *    *    *
AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC
Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys Ile Cys>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

        1180         1190         1200         1210
    *    *    *    *    *    *    *    *    *
AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA TGT
Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

  1220         1230         1240         1250         1260
    *    *    *    *    *    *    *    *    *
GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC
Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>
```

Figure 2D

```
        1270          1280          1290          1300
     *     *     *     *     *     *     *     *     *
ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA GGC TCA
Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

  1310          1320          1330          1340          1350
     *     *     *     *     *     *     *     *     *     *
GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT
Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp>
___d___d___d__ANG2 FIBRINOGEN-LIKE DOMAIN#2____d___d___d___>

        1360          1370          1380          1390
     *     *     *     *     *     *     *     *     *
TTC GGA CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC
Phe>
___>
    Gly Pro Gly>
    ___e___e___>
                Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys>
                ___f___f___f___f__FC TAG___f___f___f___f___>

  1400          1410          1420          1430          1440
     *     *     *     *     *     *     *     *     *
CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

        1450          1460          1470          1480
     *     *     *     *     *     *     *     *     *
CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

  1490          1500          1510          1520          1530
     *     *     *     *     *     *     *     *     *
CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

        1540          1550          1560          1570
     *     *     *     *     *     *     *     *     *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

  1580          1590          1600          1610          1620
     *     *     *     *     *     *     *     *     *
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

        1630          1640          1650          1660.
     *     *     *     *     *     *     *     *     *
GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>
```

Figure 2E

```
 1670          1680          1690          1700          1710
    *     *       *     *       *     *       *     *       *
AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro>
___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

         1720          1730          1740          1750
    *     *       *     *       *     *       *     *       *
ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

 1760          1770          1780          1790          1800
    *     *       *     *       *     *       *     *       *
CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

         1810          1820          1830          1840
    *     *       *     *       *     *       *     *       *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

 1850          1860          1870          1880          1890
    *     *       *     *       *     *       *     *       *
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

         1900          1910          1920          1930
    *     *       *     *       *     *       *     *       *
AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

 1940          1950          1960          1970          1980
    *     *       *     *       *     *       *     *       *
TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

         1990          2000          2010          2020
    *     *       *     *       *     *       *     *       *
GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr>
___f___f___f___f___f___f___f__FC TAG___f___f___f___f___f___f___>

 2030          2040          2050          2060
    *     *       *     *       *     *       *
ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
___f___f___f___f_____FC TAG_f___f___f___f___f___>
```

Figure 3A

```
          10          20          30          40
     *    *    *    *    *    *    *    *    *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
___a___a___a___a_TRYPSIN SIGNAL SEQUENCE___a___a___a___a___>

     50          60          70          80          90
     *    *    *    *    *    *    *    *    *
AGA GAC TGT GCA GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA
Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          100         110         120         130
     *    *    *    *    *    *    *    *    *
ATC TAC ACT ATT TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG
Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     140         150         160         170         180
     *    *    *    *    *    *    *    *    *
TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA
Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          190         200         210         220
     *    *    *    *    *    *    *    *    *
CAT CGT GAA GAT GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA
His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     230         240         250         260         270
     *    *    *    *    *    *    *    *    *
TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG
Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          280         290         300         310
     *    *    *    *    *    *    *    *    *
AAT GAG TTT ATT TTT GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA
Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     320         330         340         350         360
     *    *    *    *    *    *    *    *    *
AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG
Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          370         380         390         400
     *    *    *    *    *    *    *    *    *
TAT GAC AGA TTC CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG
Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>
```

Figure 3B

```
   410          420          430          440          450
    *    *    *    *    *    *    *    *    *
TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG

Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

         460          470          480          490
    *    *    *    *    *    *    *    *    *
ATC TTA CAC GGT GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC
Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   500          510          520          530          540
    *    *    *    *    *    *    *    *    *
AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

         550          560          570          580
    *    *    *    *    *    *    *    *    *
TTT GAT GCT TGT GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   590          600          610          620          630
    *    *    *    *    *    *    *    *    *
GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC
Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

         640          650          660          670
    *    *    *    *    *    *    *    *    *
TTC AAA GGG CCA AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT
Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile>
___b___b___b___ANG1 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   680          690          700          710          720
    *    *    *    *    *    *    *    *    *
CGA CCT TTA GAT TTT GGC CCG GGC GAG CCC AAA TCT TGT GAC AAA
Arg Pro Leu Asp Phe>
___ANG1 FIBRINO____>
                  Gly Pro Gly>
                  ___c___c___>
                              Glu Pro Lys Ser Cys Asp Lys>
                              ___d___d__FC TAG___d___d___>

         730          740          750          760
    *    *    *    *    *    *    *    *    *
ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

   770          780          790          800          810
    *    *    *    *    *    *    *    *    *
CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
```

Figure 3C

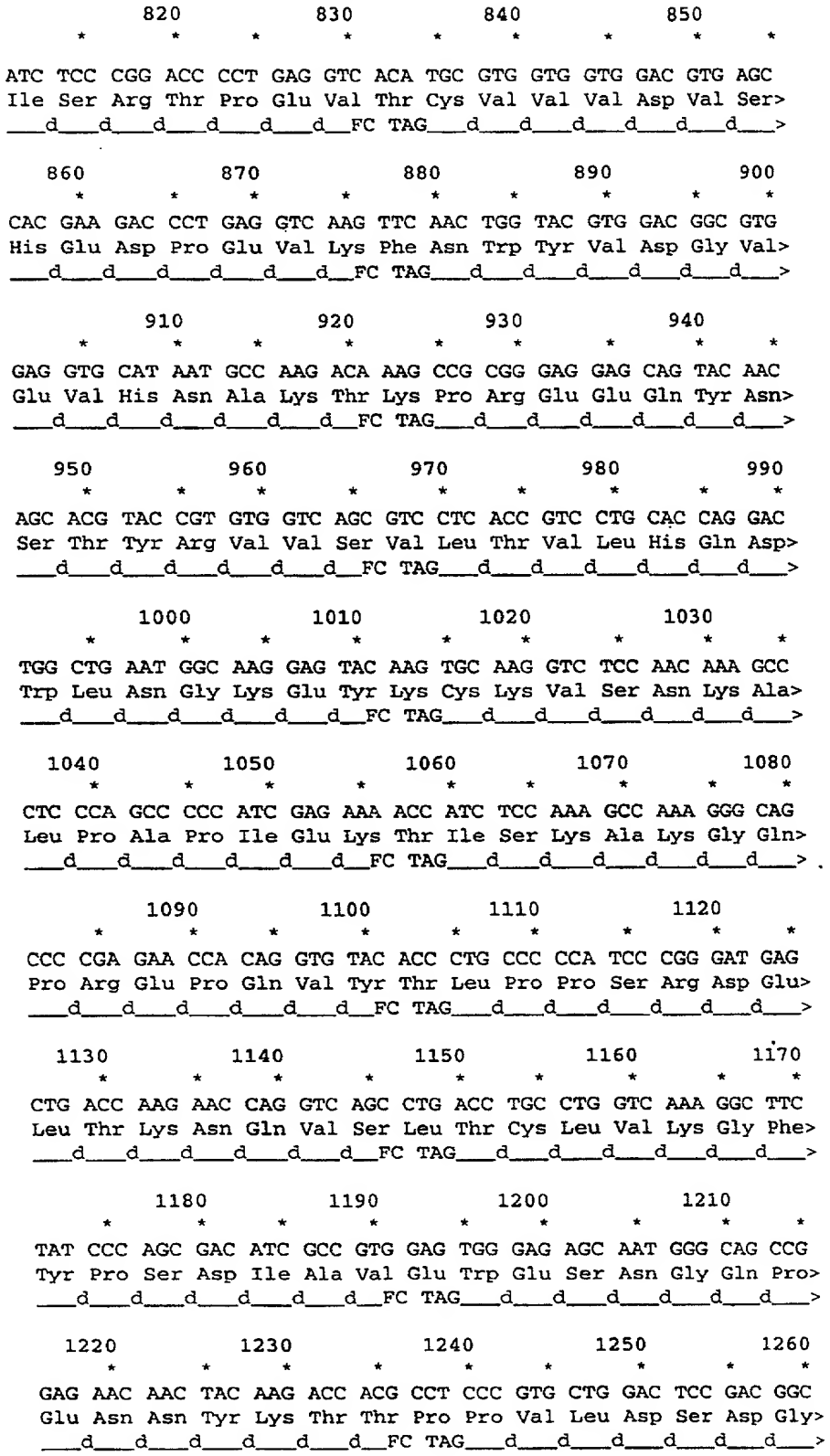

820 830 840 850
ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
860 870 880 890 900
CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
910 920 930 940
GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
950 960 970 980 990
AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1000 1010 1020 1030
TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1040 1050 1060 1070 1080
CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1090 1100 1110 1120
CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1130 1140 1150 1160 1170
CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1180 1190 1200 1210
TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1220 1230 1240 1250 1260
GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

Figure 3D

```
        1270         1280         1290         1300
     *      *     *      *      *      *      *      *     *
TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

  1310         1320         1330         1340         1350
     *      *     *      *      *      *      *      *     *
CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

         1360         1370         1380         1390
     *      *     *      *      *      *      *      *     *
CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

  1400         1410         1420         1430         1440
     *      *     *      *      *      *      *      *     *
GGC GGT GGC GGT TCT GGC GCG CCT TTT AGA GAC TGT GCA GAT GTA
Gly Gly Gly Gly Ser Gly Ala Pro>
___G4S LINKER/ASC BRIDGE (N____>
                                Phe Arg Asp Cys Ala Asp Val>
                                ___ANG1 FIBRINOGEN-LIKE____>

         1450         1460         1470         1480
     *      *     *      *      *      *      *      *     *
TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT ATT
Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1490         1500         1510         1520         1530
     *      *     *      *      *      *      *      *     *
AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC
Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

         1540         1550         1560         1570
     *      *     *      *      *      *      *      *     * *
AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA AGT
Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1580         1590         1600         1610         1620
     *      *     *      *      *      *      *      *     *
CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA
Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

         1630         1640         1650         1660
     *      *     *      *      *      *      *      *     *
AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT GCC
Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>
```

Figure 3E

```
  1670         1680          1690         1700         1710
    *      *     *      *      *      *      *      *      *
ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

         1720         1730         1740         1750
     *      *     *      *      *      *      *      *      *
TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC ATA
Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1760         1770          1780         1790         1800
    *      *     *      *      *      *      *      *      *
GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT
Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

         1810         1820         1830         1840
     *      *     *      *      *      *      *      *      *
GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT GCT GAT
Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1850         1860          1870         1880         1890
    *      *     *      *      *      *      *      *      *
TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA TGT
Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

         1900         1910         1920         1930
     *      *     *      *      *      *      *      *      *
GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC CCC
Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1940         1950          1960         1970         1980
    *      *     *      *      *      *      *      *      *
TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT GGA
Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His Gly>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

         1990         2000         2010         2020
     *      *     *      *      *      *      *      *      *
AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCA AGT TAC
Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser Tyr>
___f___f___f___ANG1 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  2030         2040          2050         2060
    *      *     *      *      *      *      *      *
TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT
Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe>
___f___f___f_ANG1 FIBRINOGEN-LIKE DOMAIN___f___f___f___>
```

Figure 4A

```
          10          20          30          40
     *    *    *    *    *    *    *    *    *
ATG TCT GCA CTT CTG ATC CTA GCT CTT GTT GGA GCT GCA GTT GCT
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala>
___a___a___a___a_TRYPSIN SIGNAL SEQUENCE___a___a___a___a___>

     50          60          70          80          90
     *    *    *    *    *    *    *    *    *
AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC
Arg Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          100         110         120         130
     *    *    *    *    *    *    *    *    *
ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC
Ile Tyr Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     140         150         160         170         180
     *    *    *    *    *    *    *    *    *
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG
Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          190         200         210         220
     *    *    *    *    *    *    *    *    *
CGA CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA
Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     230         240         250         260         270
     *    *    *    *    *    *    *    *    *
TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA
Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          280         290         300         310
     *    *    *    *    *    *    *    *    *
AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT
Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr Val Leu>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

     320         330         340         350         360
     *    *    *    *    *    *    *    *    *
AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG
Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser Leu>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          370         380         390         400
     *    *    *    *    *    *    *    *    *
TAT GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT
Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>
```

Figure 4B

```
   410          420          430          440          450
    *    *    *    *    *    *    *    *    *
CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC

His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          460          470          480          490
    *    *    *    *    *    *    *    *    *
AGC CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC
Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   500          510          520          530          540
    *    *    *    *    *    *    *    *    *
AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG
Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          550          560          570          580
    *    *    *    *    *    *    *    *    *
TTT GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   590          600          610          620          630
    *    *    *    *    *    *    *    *    *
CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

          640          650          660          670
    *    *    *    *    *    *    *    *    *
TGG AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC
Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile>
___b___b___b___ANG2 FIBRINOGEN-LIKE DOMAIN_b___b___b___b___>

   680          690          700          710          720
    *    *    *    *    *    *    *    *    *
CGA CCA GCA GAT TTC GGG GGC CCG GGC GAG CCC AAA TCT TGT GAC
Arg Pro Ala Asp Phe>
___ANG2 FIBRINO____>
                  Gly Gly Pro Gly>
                  ___GGPG BRI____>
                                  Glu Pro Lys Ser Cys Asp>
                                  ___d____FC TAG_d___d___>

          730          740          750          760
    *    *    *    *    *    *    *    *    *
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

   770          780          790          800          810
    *    *    *    *    *    *    *    *    *
GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
```

Figure 4C

```
          820         830         840         850
      *     *     *     *     *     *     *     *     *
ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

   860         870         880         890         900
      *     *     *     *     *     *     *     *     *
AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

          910         920         930         940
      *     *     *     *     *     *     *     *     *
GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

   950         960         970         980         990
      *     *     *     *     *     *     *     *     *
AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

         1000        1010        1020        1030
      *     *     *     *     *     *     *     *     *
GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

  1040        1050        1060        1070        1080
      *     *     *     *     *     *     *     *     *
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

         1090        1100        1110        1120
      *     *     *     *     *     *     *     *     *
CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

  1130        1140        1150        1160        1170
      *     *     *     *     *     *     *     *     *
GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

         1180        1190        1200        1210
      *     *     *     *     *     *     *     *     *
TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>

  1220        1230        1240        1250        1260
      *     *     *     *     *     *     *     *     *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
```

Figure 4D

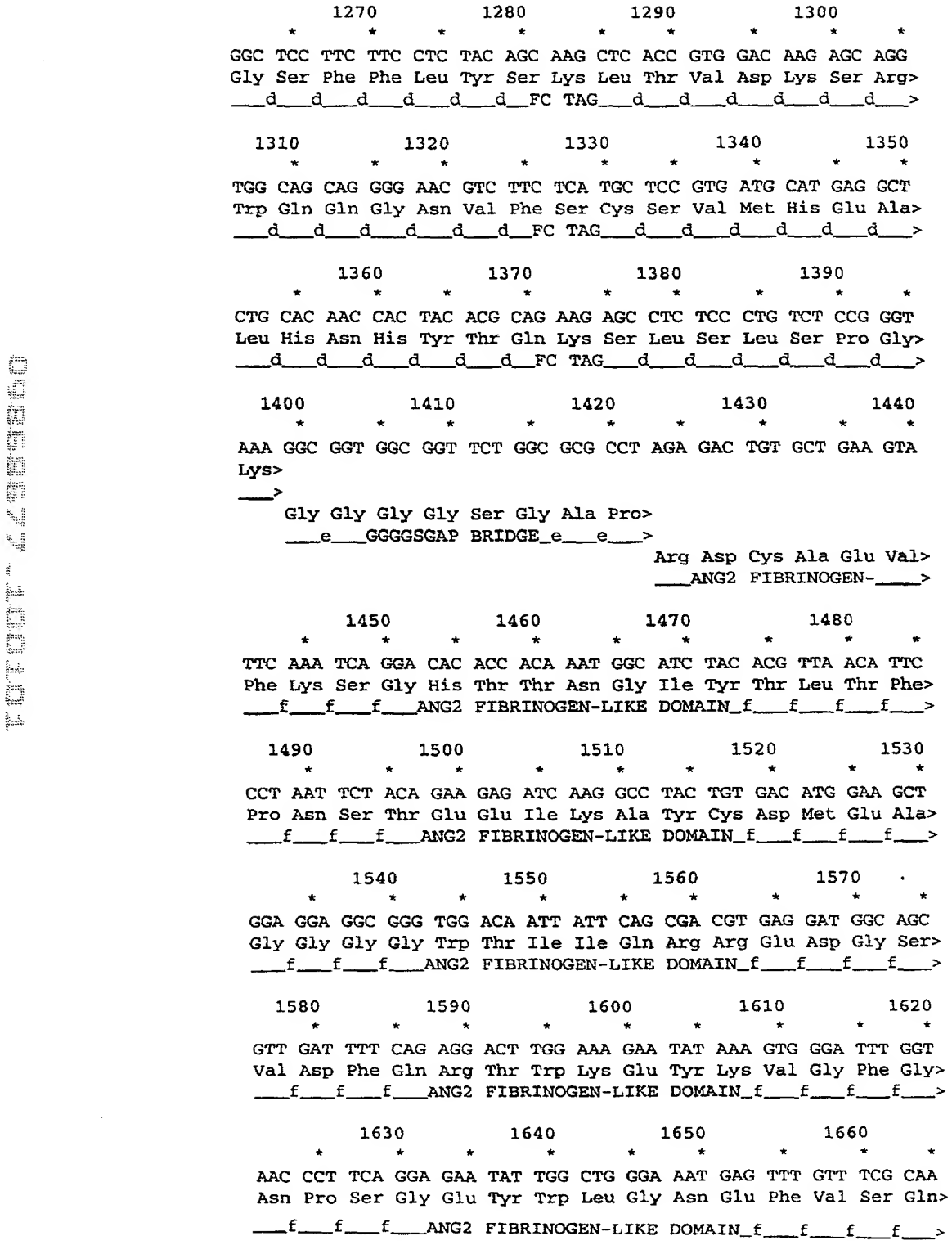

1270 1280 1290 1300
GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1310 1320 1330 1340 1350
TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1360 1370 1380 1390
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly>
___d___d___d___d___d___d__FC TAG___d___d___d___d___d___d___>
1400 1410 1420 1430 1440
AAA GGC GGT GGC GGT TCT GGC GCG CCT AGA GAC TGT GCT GAA GTA
Lys>
___>
Gly Gly Gly Gly Ser Gly Ala Pro>
___e___GGGGSGAP BRIDGE_e___e___>
Arg Asp Cys Ala Glu Val>
___ANG2 FIBRINOGEN-____>
1450 1460 1470 1480
TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC ACG TTA ACA TTC
Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>
1490 1500 1510 1520 1530
CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT
Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>
1540 1550 1560 1570
GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC AGC
Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>
1580 1590 1600 1610 1620
GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT
Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>
1630 1640 1650 1660
AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT GAG TTT GTT TCG CAA
Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

Figure 4E

```
  1670          1680          1690          1700          1710
     *       *     *       *     *       *     *       *     *
CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

          1720          1730          1740          1750
     *       *     *       *     *       *     *       *     *
TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC TAT CTC
Trp Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1760          1770          1780          1790          1800
     *       *     *       *     *       *     *       *     *
TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA
Ser Ser Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

          1810          1820          1830          1840
     *       *     *       *     *       *     *       *     *
GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC CAA CCA GGA AAT GAT
Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1850          1860          1870          1880          1890
     *       *     *       *     *       *     *       *     *
TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC AAA TGT
Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

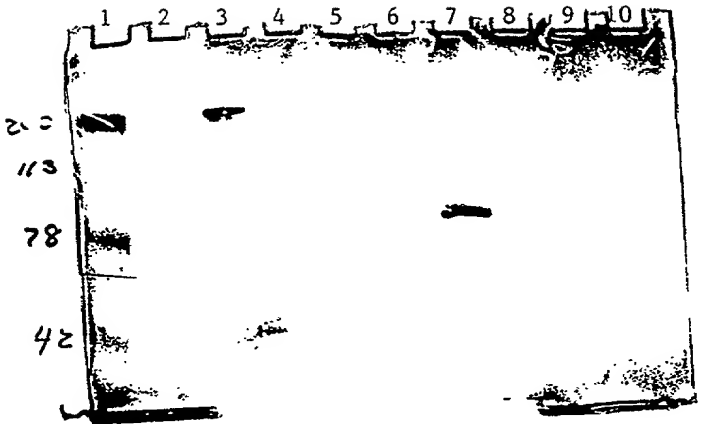
          1900          1910          1920          1930
     *       *     *       *     *       *     *       *     *
TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA TGT GGT CCT
Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  1940          1950          1960          1970          1980
     *       *     *       *     *       *     *       *     *
TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT
Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn Thr Asn>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

          1990          2000          2010          2020
     *       *     *       *     *       *     *       *     *
AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA GGC TCA GGC TAT
Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser Gly Tyr>
___f___f___f___ANG2 FIBRINOGEN-LIKE DOMAIN_f___f___f___f___>

  2030          2040          2050          2060          2070
     *       *     *       *     *       *     *       *     *
TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT TTC TGA
Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe>
___f___f___f_ANG2 FIBRINOGEN-LIKE DOMAIN___f___f___f___>
```

Figure 5



Angl-FD-Fc-FD

Figure 6

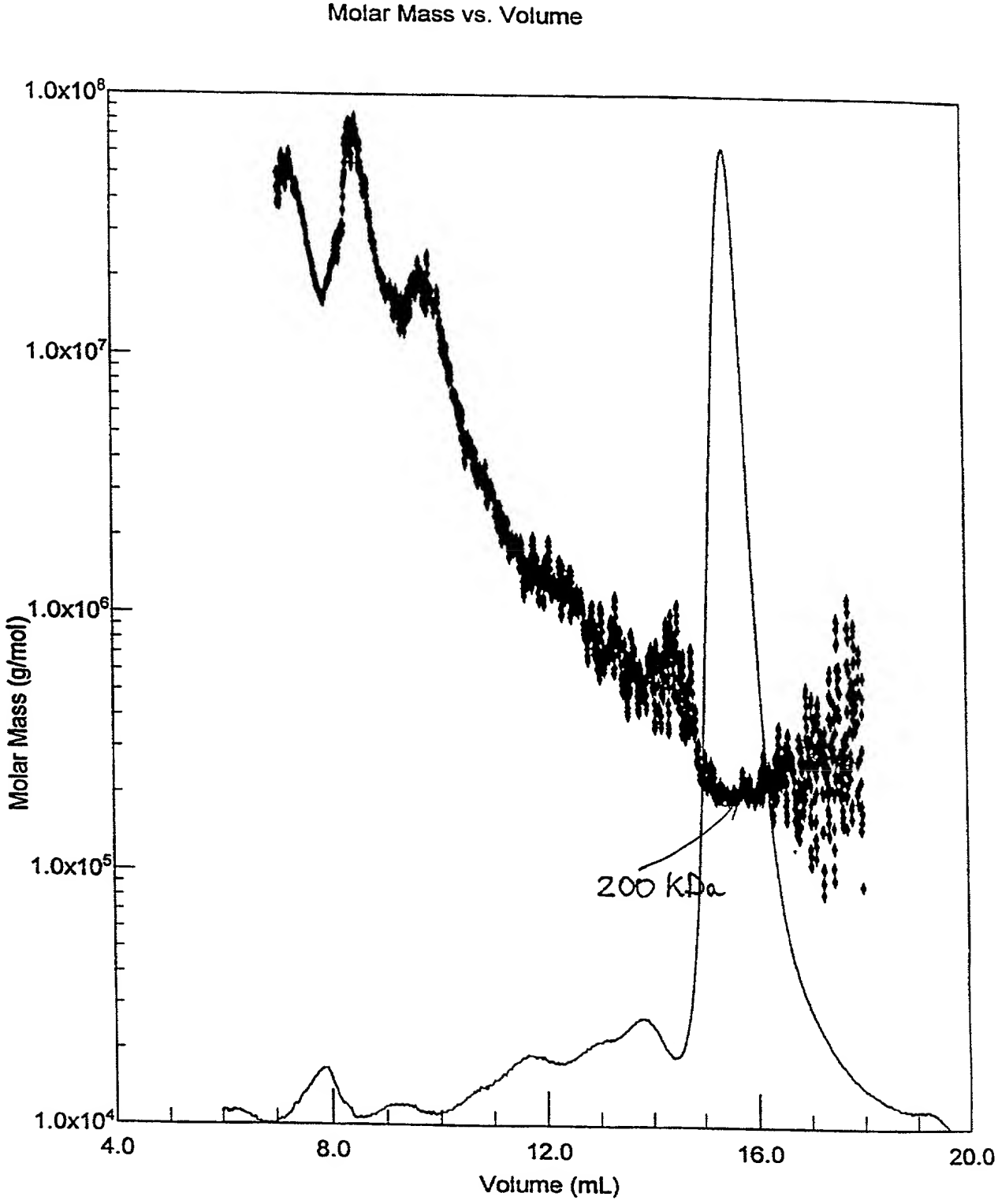
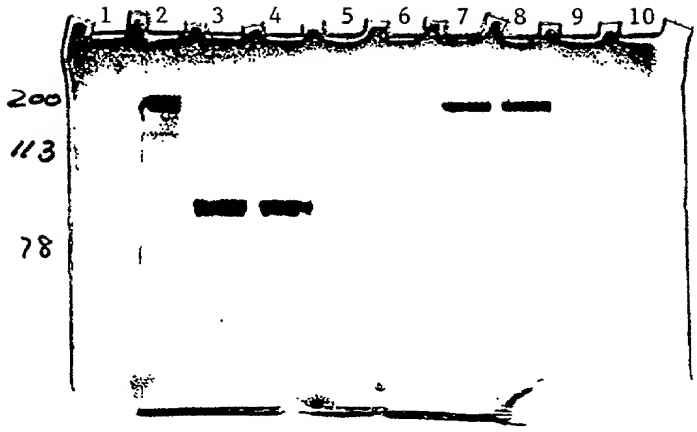

Molar Mass vs. Volume
1.0x10^8
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
Molar Mass (g/mol)
200 KDa
4.0
8.0
12.0
16.0
20.0
Volume (mL)

Figure 7



Ang2-FD-Fc-FD

Figure 8

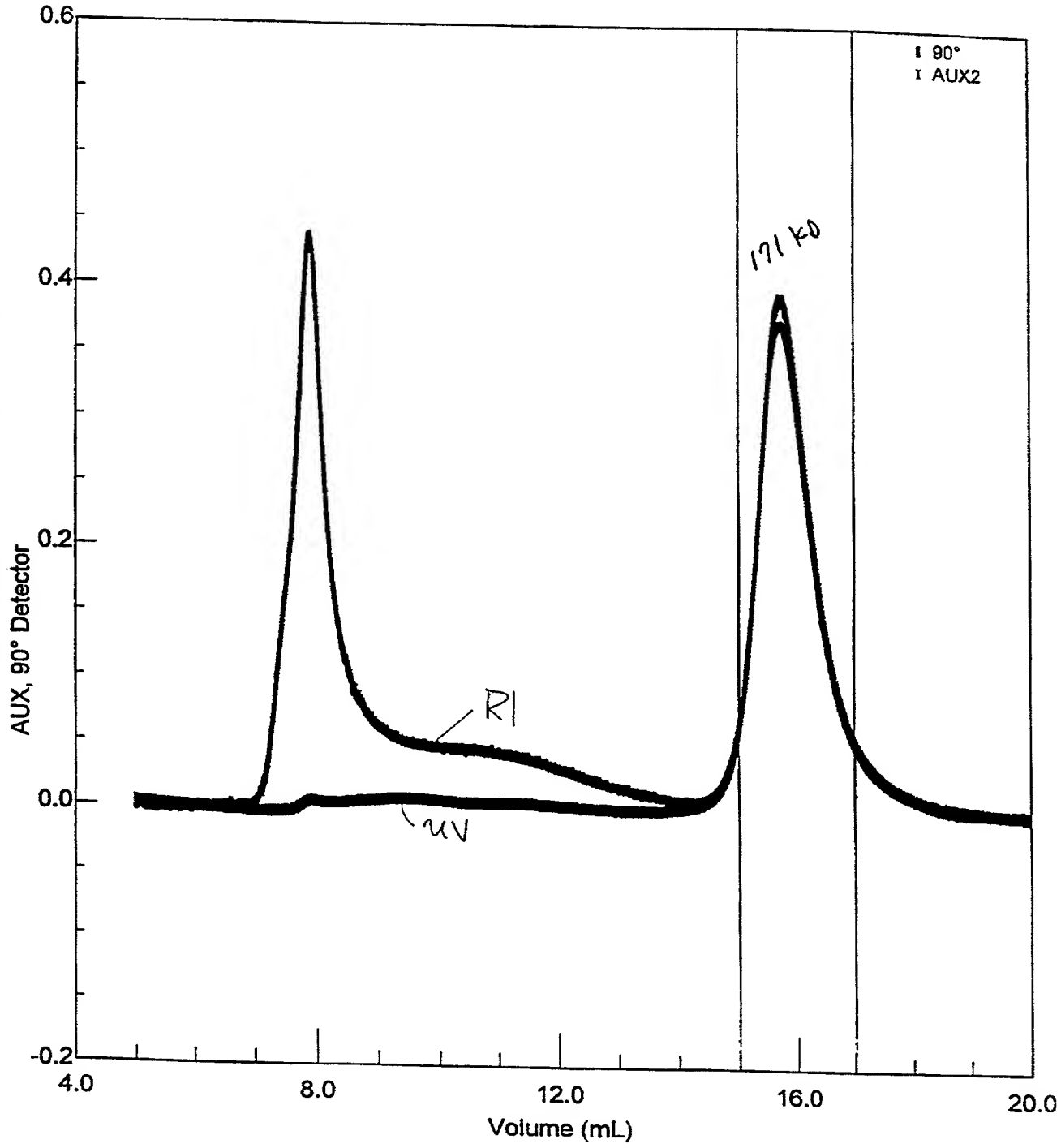

0.6
0.4
0.2
0.0
-0.2
AUX, 90° Detector
4.0
8.0
12.0
16.0
20.0
Volume (mL)
90°
AUX2
171 KD
RI
UV

Figure 9

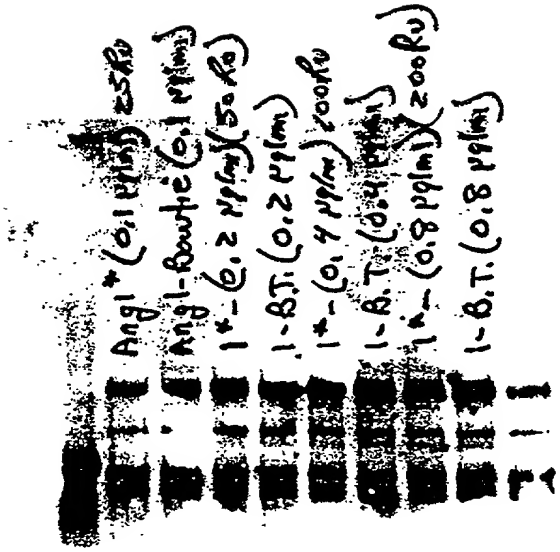

Angl + (0,1 μg/ml) 25kRu
Angl-Bouche (0,1 μg/ml)
1+ - (0,2 μg/ml) (50 Ru)
1-B.T. (0,2 μg/ml)
1+ - (0,4 μg/ml) 200Ru
1-B.T. (0,4 μg/ml)
1+ - (0,8 μg/ml) 200Ru
1-B.T. (0,8 μg/ml)
1 2 3 4 5 6 7 8 9 10

Figure 10

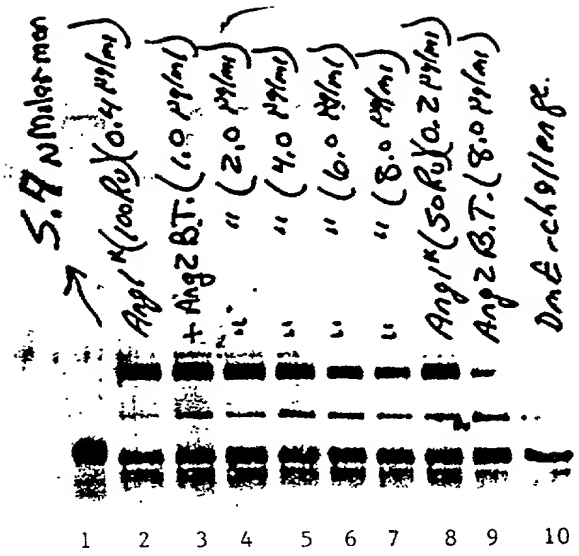

5.9 N Molar mass
Ang I (100 Pu) (0.4 μg/ml)
+ Ang 2 B.T. (1.0 μg/ml)
" (2.0 μg/ml)
" (4.0 μg/ml)
" (6.0 μg/ml)
" (8.0 μg/ml)
Ang I (50 Pu) (0.2 μg/ml)
Ang 2 B.T. (8.0 μg/ml)
DMF-chloroform
1 2 3 4 5 6 7 8 9 10

Figure 11

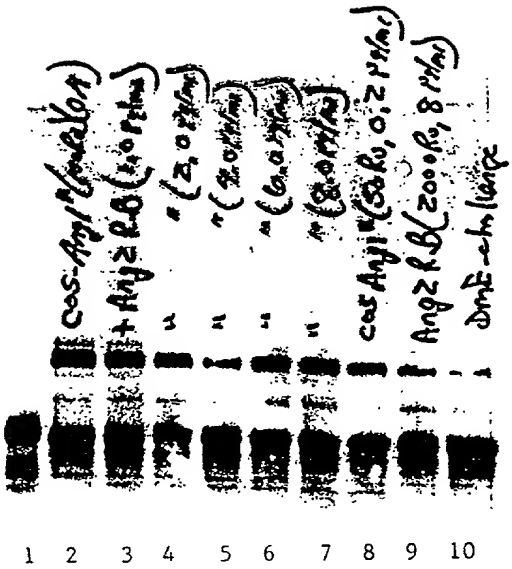


Figure 12

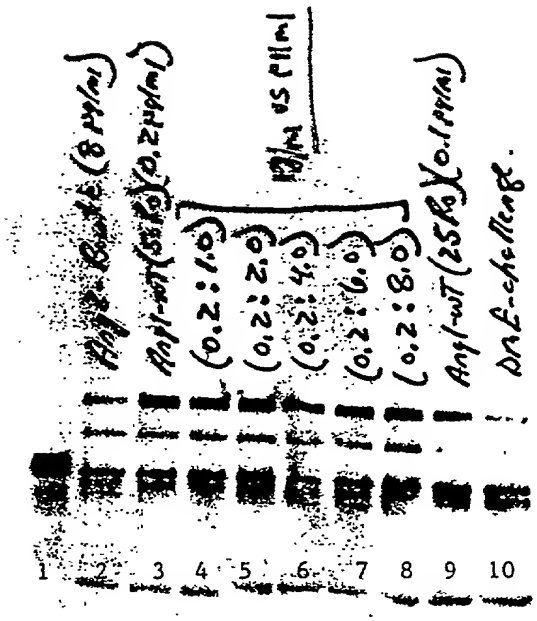

Hyal + Bovine (8 mg/ml)
Hyal + Ang-I-uT (25 Ro)(0.2 mg/ml)
(0.2 : 1.0)
(0.2 : 2.0)
(0.2 : 4.0)
(0.2 : 6.0)
(0.2 : 8.0)
IN vs PIIINI
Ang-I-uT (25 Ro)(0.1 mg/ml)
DNL-chelerythr.
1 2 3 4 5 6 7 8 9 10

Figure 13

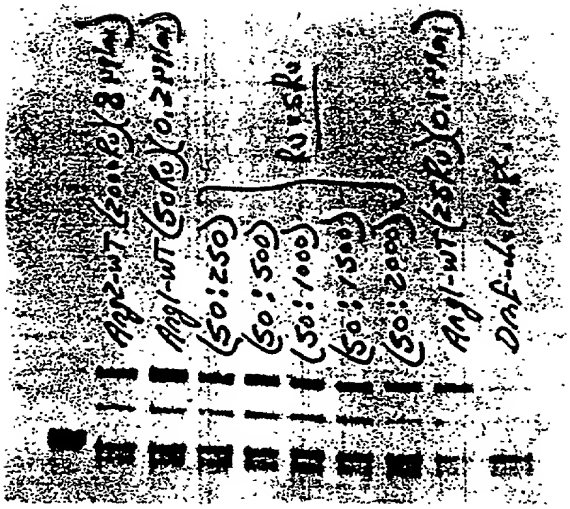

Arg2-wT (20:1) (8 mM)
Arg1-wT (50:1) (2 mM)
(50:250)
(50:500)
(50:1000)
RU1SKb
(50:1500)
(50:2000)
Arg1-wT (250:1) (1 mM)
Draf-1 (1 mM)
1 2 3 4 5 6 7 8 9 10

Figure 14A

```
          10          20          30          40
     *    *    *    *    *    *    *    *    *
ATG GCT CGG CCT GGG CAG CGT TGG CTC GGC AAG TGG CTT GTG GCG
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

     50          60          70          80          90
     *    *    *    *    *    *    *    *    *
ATG GTC GTG TGG GCG CTG TGC CGG CTC GCC ACA CCG CTG GCC AAG
Met Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

         100         110         120         130
     *    *    *    *    *    *    *    *    *
AAC CTG GAG CCC GTA TCC TGG AGC TCC CTC AAC CCC AAG TTC CTG
Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

    140         150         160         170         180
     *    *    *    *    *    *    *    *    *
AGT GGG AAG GGC TTG GTG ATC TAT CCG AAA ATT GGA GAC AAG CTG
Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

          190         200         210         220
     *    *    *    *    *    *    *    *    *
GAC ATC ATC TGC CCC CGA GCA GAA GCA GGG CGG CCC TAT GAG TAC
Asp Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

    230         240         250         260         270
     *    *    *    *    *    *    *    *    *
TAC AAG CTG TAC CTG GTG CGG CCT GAG CAG GCA GCT GCC TGT AGC
Tyr Lys Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Ala Cys Ser>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

          280         290         300         310
     *    *    *    *    *    *    *    *    *
ACA GTT CTC GAC CCC AAC GTG TTG GTC ACC TGC AAT AGG CCA GAG
Thr Val Leu Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro Glu>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

    320         330         340         350         360
     *    *    *    *    *    *    *    *    *
CAG GAA ATA CGC TTT ACC ATC AAG TTC CAG GAG TTC AGC CCC AAC
Gln Glu Ile Arg Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>

          370         380         390         400
     *    *    *    *    *    *    *    *    *
TAC ATG GGC CTG GAG TTC AAG AAG CAC CAT GAT TAC TAC ATT ACC
Tyr Met Gly Leu Glu Phe Lys Lys His His Asp Tyr Tyr Ile Thr>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
```

Figure 14B

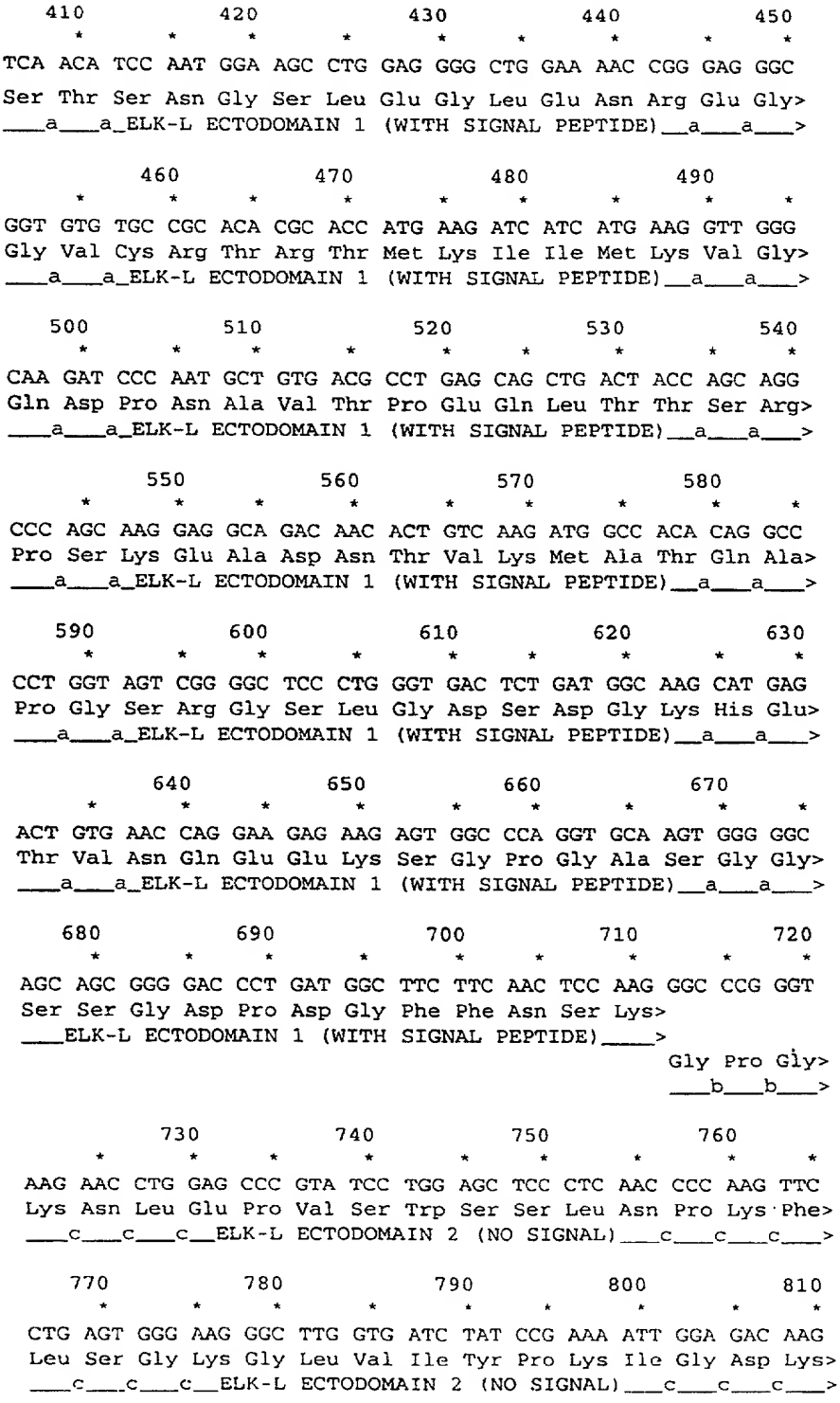

410 420 430 440 450
TCA ACA TCC AAT GGA AGC CTG GAG GGG CTG GAA AAC CGG GAG GGC
Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Glu Asn Arg Glu Gly>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
460 470 480 490
GGT GTG TGC CGC ACA CGC ACC ATG AAG ATC ATC ATG AAG GTT GGG
Gly Val Cys Arg Thr Arg Thr Met Lys Ile Ile Met Lys Val Gly>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
500 510 520 530 540
CAA GAT CCC AAT GCT GTG ACG CCT GAG CAG CTG ACT ACC AGC AGG
Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu Thr Thr Ser Arg>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
550 560 570 580
CCC AGC AAG GAG GCA GAC AAC ACT GTC AAG ATG GCC ACA CAG GCC
Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala Thr Gln Ala>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
590 600 610 620 630
CCT GGT AGT CGG GGC TCC CTG GGT GAC TCT GAT GGC AAG CAT GAG
Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys His Glu>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
640 650 660 670
ACT GTG AAC CAG GAA GAG AAG AGT GGC CCA GGT GCA AGT GGG GGC
Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly Gly>
___a___a_ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)__a___a___>
680 690 700 710 720
AGC AGC GGG GAC CCT GAT GGC TTC TTC AAC TCC AAG GGC CCG GGT
Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys>
___ELK-L ECTODOMAIN 1 (WITH SIGNAL PEPTIDE)____>
Gly Pro Gly>
___b___b___>
730 740 750 760
AAG AAC CTG GAG CCC GTA TCC TGG AGC TCC CTC AAC CCC AAG TTC
Lys Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>
770 780 790 800 810
CTG AGT GGG AAG GGC TTG GTG ATC TAT CCG AAA ATT GGA GAC AAG
Leu Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>
SUBSTITUTE SHEET (RULE 26)

Figure 14C

```
        820         830         840         850
    *   *   *   *   *   *   *   *   *   *   *
CTG GAC ATC ATC TGC CCC CGA GCA GAA GCA GGG CGG CCC TAT GAG
Leu Asp Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

   860         870         880         890         900
    *   *   *   *   *   *   *   *   *   *   *
TAC TAC AAG CTG TAC CTG GTG CGG CCT GAG CAG GCA GCT GCC TGT
Tyr Tyr Lys Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Ala Cys>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

        910         920         930         940
    *   *   *   *   *   *   *   *   *   *   *
AGC ACA GTT CTC GAC CCC AAC GTG TTG GTC ACC TGC AAT AGG CCA
Ser Thr Val Leu Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

   950         960         970         980         990
    *   *   *   *   *   *   *   *   *   *   *
GAG CAG GAA ATA CGC TTT ACC ATC AAG TTC CAG GAG TTC AGC CCC
Glu Gln Glu Ile Arg Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

       1000        1010        1020        1030
    *   *   *   *   *   *   *   *   *   *   *
AAC TAC ATG GGC CTG GAG TTC AAG AAG CAC CAT GAT TAC TAC ATT
Asn Tyr Met Gly Leu Glu Phe Lys Lys His His Asp Tyr Tyr Ile>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

  1040        1050        1060        1070        1080
    *   *   *   *   *   *   *   *   *   *   *
ACC TCA ACA TCC AAT GGA AGC CTG GAG GGG CTG GAA AAC CGG GAG
Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Glu Asn Arg Glu>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

        1090        1100        1110        1120
    *   *   *   *   *   *   *   *   *   *   *
GGC GGT GTG TGC CGC ACA CGC ACC ATG AAG ATC ATC ATG AAG GTT
Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile Ile Met Lys Val>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

  1130        1140        1150        1160        1170
    *   *   *   *   *   *   *   *   *   *   *
GGG CAA GAT CCC AAT GCT GTG ACG CCT GAG CAG CTG ACT ACC AGC
Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu Thr Thr Ser>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

        1180        1190        1200        1210
    *   *   *   *   *   *   *   *   *   *   *
AGG CCC AGC AAG GAG GCA GAC AAC ACT GTC AAG ATG GCC ACA CAG
Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala Thr Gln>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>

  1220        1230        1240        1250        1260
    *   *   *   *   *   *   *   *   *   *   *
GCC CCT GGT AGT CGG GGC TCC CTG GGT GAC TCT GAT GGC AAG CAT
Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys His>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>
```

Figure 14D

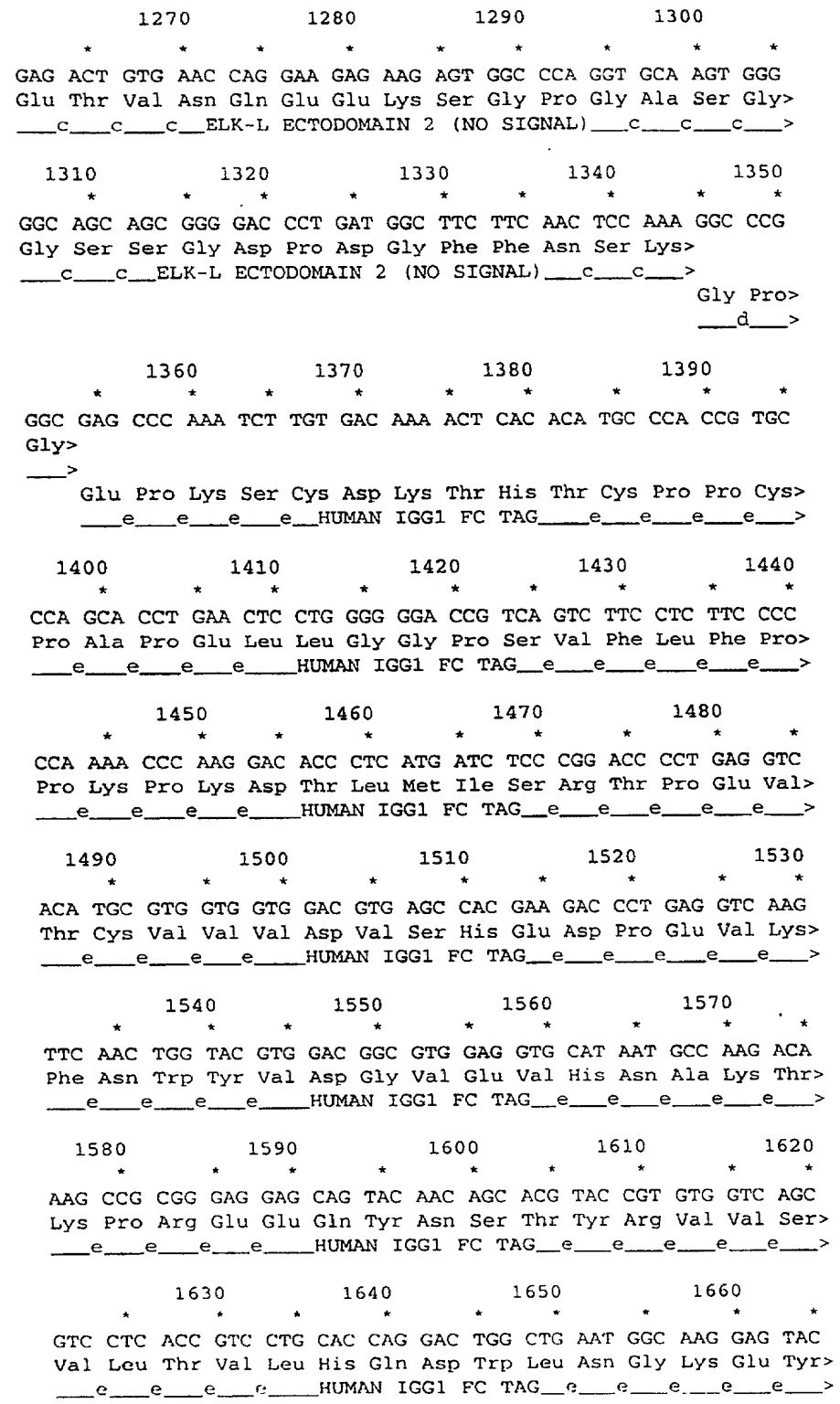

1270 1280 1290 1300
GAG ACT GTG AAC CAG GAA GAG AAG AGT GGC CCA GGT GCA AGT GGG
Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly>
___c___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___c___>
1310 1320 1330 1340 1350
GGC AGC AGC GGG GAC CCT GAT GGC TTC TTC AAC TCC AAA GGC CCG
Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys>
___c___c__ELK-L ECTODOMAIN 2 (NO SIGNAL)___c___c___>
Gly Pro>
___d___>
1360 1370 1380 1390
GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC
Gly>
___>
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys>
___e___e___e___e__HUMAN IGG1 FC TAG____e___e___e___e___>
1400 1410 1420 1430 1440
CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>
1450 1460 1470 1480
CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>
1490 1500 1510 1520 1530
ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>
1540 1550 1560 1570
TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>
1580 1590 1600 1610 1620
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>
1630 1640 1650 1660
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

Figure 14E

```
 1670         1680          1690          1700          1710
    *     *     *     *     *     *     *     *     *     *
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

        1720          1730          1740          1750
    *     *     *     *     *     *     *     *     *
ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

 1760          1770          1780          1790          1800
    *     *     *     *     *     *     *     *     *
ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

        1810          1820          1830          1840
    *     *     *     *     *     *     *     *     *
CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

 1850          1860          1870          1880          1890
    *     *     *     *     *     *     *     *     *
GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

        1900          1910          1920          1930
    *     *     *     *     *     *     *     *     *
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

 1940          1950          1960          1970          1980
    *     *     *     *     *     *     *     *     *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

        1990          2000          2010          2020
    *     *     *     *     *     *     *     *     *
TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys>
___e___e___e___e____HUMAN IGG1 FC TAG__e___e___e___e___e___>

 2030          2040          2050
    *     *     *     *     *
AGC CTC TCC CTG TCT CCG GGT AAA TGA
Ser Leu Ser Leu Ser Pro Gly Lys ***>
___e____HUMAN IGG1 FC TAG__e___e___>
```

Figure 15A

```
          10          20          30          40
     *    *     *     *     *     *     *     *     *
ATG GCC ATG GCC CGG TCC AGG AGG GAC TCT GTG TGG AAG TAC TGT
Met Ala Met Ala Arg Ser Arg Arg Asp Ser Val Trp Lys Tyr Cys>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

    50          60          70          80          90
     *    *     *     *     *     *     *     *     *
TGG GGA CTT TTG ATG GTT TTG TGC AGA ACT GCG ATC TCC AGA TCG
Trp Gly Leu Leu Met Val Leu Cys Arg Thr Ala Ile Ser Arg Ser>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

         100         110         120         130
     *    *     *     *     *     *     *     *     *
ATA GTT TTA GAG CCT ATC TAC TGG AAT TCC TCG AAC TCC AAA TTT
Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn Ser Lys Phe>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

   140         150         160         170         180
     *    *     *     *     *     *     *     *     *
CTA CCC GGA CAA GGC CTG GTA CTA TAC CCA CAG ATA GGA GAC AAA
Leu Pro Gly Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly Asp Lys>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

         190         200         210         220
     *    *     *     *     *     *     *     *     *
TTG GAT ATT ATT TGC CCC AAA GTG GAC TCT AAA ACT GTT GGC CAG
Leu Asp Ile Ile Cys Pro Lys Val Asp Ser Lys Thr Val Gly Gln>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

   230         240         250         260         270
     *    *     *     *     *     *     *     *     *
TAT GAA TAT TAT AAA GTT TAT ATG GTT GAT AAA GAC CAA GCA GAC
Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp Gln Ala Asp>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

         280         290         300         310
     *    *     *     *     *     *     *     *     *
AGA TGC ACA ATT AAG AAG GAG AAT ACC CCG CTG CTC AAC TGT GCC
Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu Asn Cys Ala>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

   320         330         340         350         360
     *    *     *     *     *     *     *     *     *
AGA CCA GAC CAA GAT GTG AAA TTC ACC ATC AAG TTT CAA GAA TTC
Arg Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe Gln Glu Phe>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

         370         380         390         400
     *    *     *     *     *     *     *     *     *
AGC CCT AAC CTC TGG GGT CTA GAA TTT CAG AAG AAC AAA GAT TAC
Ser Pro Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn Lys Asp Tyr>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>
```

Figure 15B

```
   410          420          430          440          450
    *    *    *    *    *    *    *    *    *
TAC ATT ATA TCT ACA TCA AAT GGG TCT TTG GAG GGC CTG GAT AAC
Tyr Ile Ile Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Asp Asn>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

          460          470          480          490
    *    *    *    *    *    *    *    *    *
CAG GAG GGA GGG GTG TGC CAG ACA AGA GCC ATG AAG ATC CTC ATG
Gln Glu Gly Gly Val Cys Gln Thr Arg Ala Met Lys Ile Leu Met>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

   500          510          520          530          540
    *    *    *    *    *    *    *    *    *
AAA GTT GGA CAA GAT GCA AGT TCT GCT GGA TCA GCC AGG AAT CAC
Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser Ala Arg Asn His>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

          550          560          570          580
    *    *    *    *    *    *    *    *    *
GGT CCA ACA AGA CGT CCA GAG CTA GAA GCT GGT ACA AAT GGG AGA
Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr Asn Gly Arg>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

   590          600          610          620          630
    *    *    *    *    *    *    *    *    *
AGT TCA ACA ACA AGT CCC TTT GTG AAG CCA AAT CCA GGT TCT AGC
Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro Gly Ser Ser>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

          640          650          660          670
    *    *    *    *    *    *    *    *    *
ACC GAT GGC AAC AGC GCG GGG CAT TCC GGG AAC AAT CTC CTG GGG
Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn Leu Leu Gly>
___a__EPHRIN-B2  ECTO DOMAIN 1 (WITH SIGNAL PEPTIDE)___a___>

   680          690          700          710          720
    *    *    *    *    *    *    *    *    *
GGC CCG GGA ATA GTT TTA GAG CCT ATC TAC TGG AAT TCC TCG AAC
Gly Pro Gly>
___b___b___>
              Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn>
              ___EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNA_____>

          730          740          750          760
    *    *    *    *    *    *    *    *    *
TCC AAA TTT CTA CCC GGA CAA GGC CTG GTA CTA TAC CCA CAG ATA
Ser Lys Phe Leu Pro Gly Gln Gly Leu Val Leu Tyr Pro Gln Ile>
_____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>

   770          780          790          800          810
    *    *    *    *    *    *    *    *    *
GGA GAC AAA TTG GAT ATT ATT TGC CCC AAA GTG GAC TCT AAA ACT
Gly Asp Lys Leu Asp Ile Ile Cys Pro Lys Val Asp Ser Lys Thr>
_____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e____>
```

Figure 15C

```
          820         830         840         850
     *    *    *    *    *    *    *    *    *
GTT GGC CAG TAT GAA TAT TAT AAA GTT TAT ATG GTT GAT AAA GAC
Val Gly Gln Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

   860         870         880         890         900
     *    *    *    *    *    *    *    *    *    *
CAA GCA GAC AGA TGC ACA ATT AAG AAG GAG AAT ACC CCG CTG CTC
Gln Ala Asp Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

          910         920         930         940
     *    *    *    *    *    *    *    *    *
AAC TGT GCC AGA CCA GAC CAA GAT GTG AAA TTC ACC ATC AAG TTT
Asn Cys Ala Arg Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

   950         960         970         980         990
     *    *    *    *    *    *    *    *    *
CAA GAA TTC AGC CCT AAC CTC TGG GGT CTA GAA TTT CAG AAG AAC
Gln Glu Phe Ser Pro Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

         1000        1010        1020        1030
     *    *    *    *    *    *    *    *    *
AAA GAT TAC TAC ATT ATA TCT ACA TCA AAT GGG TCT TTG GAG GGC
Lys Asp Tyr Tyr Ile Ile Ser Thr Ser Asn Gly Ser Leu Glu Gly>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

  1040        1050        1060        1070        1080
     *    *    *    *    *    *    *    *    *
CTG GAT AAC CAG GAG GGA GGG GTG TGC CAG ACA AGA GCC ATG AAG
Leu Asp Asn Gln Glu Gly Gly Val Cys Gln Thr Arg Ala Met Lys>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

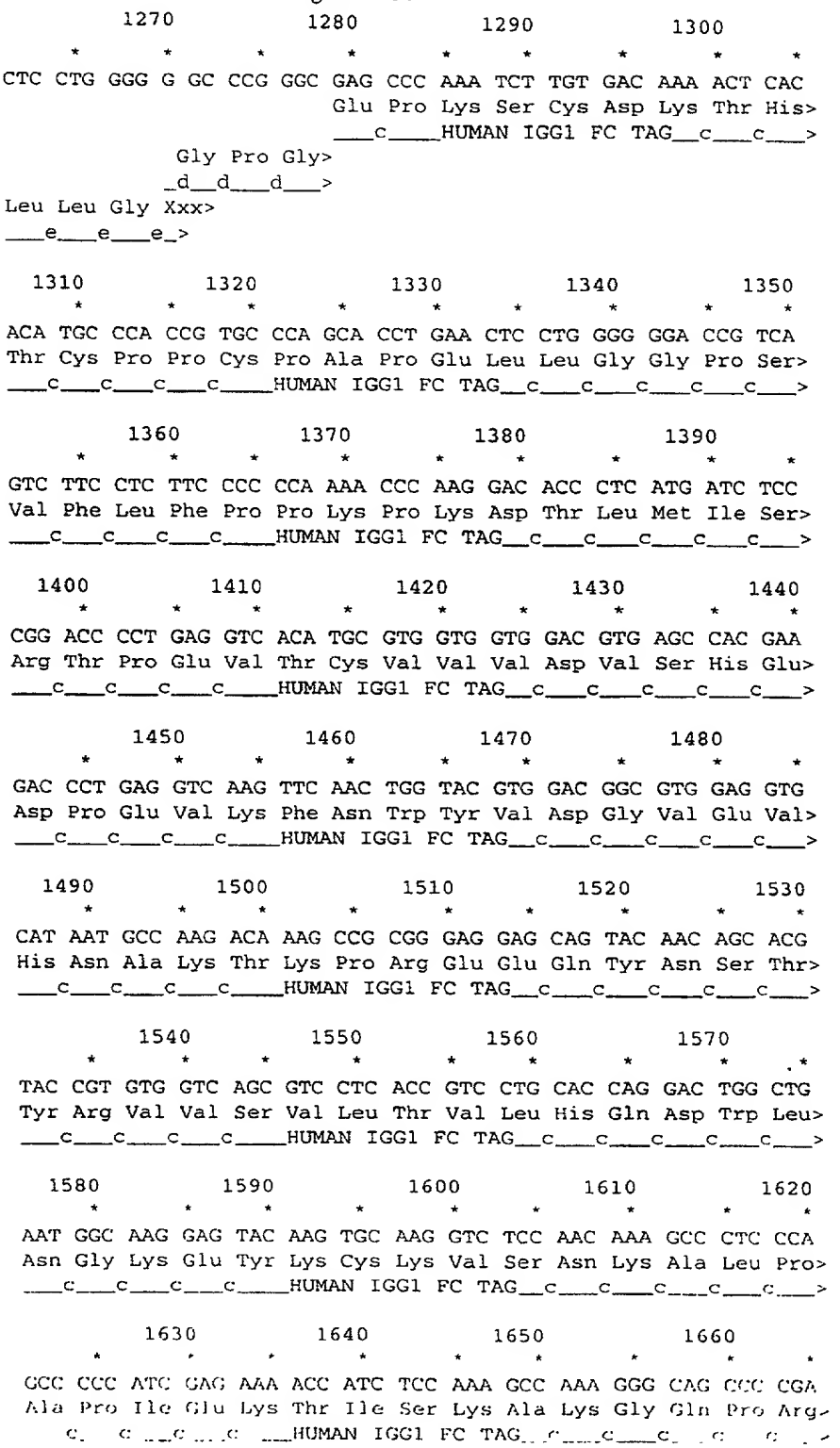
         1090        1100        1110        1120
     *    *    *    *    *    *    *    *    *
ATC CTC ATG AAA GTT GGA CAA GAT GCA AGT TCT GCT GGA TCA GCC
Ile Leu Met Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser Ala>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

  1130        1140        1150        1160        1170
     *    *    *    *    *    *    *    *    *
AGG AAT CAC GGT CCA ACA AGA CGC CCA GAG CTA GAA GCT GGT ACA
Arg Asn His Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

         1180        1190        1200        1210
     *    *    *    *    *    *    *    *    *
AAT GGG AGA AGT TCA ACA ACA AGT CCC TTT GTG AAG CCA AAT CCA
Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE)_e___>

  1220        1230        1240        1250        1260
     *    *    *    *    *    *    *    *    *
GGT TCT AGC ACC GAT GGC AAC AGC GCG GGG CAT TCC GGG AAC AAT
Gly Ser Ser Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn>
____EPHRIN-B2  ECTO DOMAIN 2 ( WITHOUT SIGNAL PEPTIDE) e___>
```

Figure 15D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CTC CTG GGG G GC CCG GGC GAG CCC AAA TCT TGT GAC AAA ACT CAC
Glu Pro Lys Ser Cys Asp Lys Thr His>
___c____HUMAN IGG1 FC TAG__c___c___>
Gly Pro Gly>
_d__d___d___>
Leu Leu Gly Xxx>
___e___e___e_>
1310 1320 1330 1340 1350
ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1360 1370 1380 1390
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1400 1410 1420 1430 1440
CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1450 1460 1470 1480
GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1490 1500 1510 1520 1530
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1540 1550 1560 1570
TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1580 1590 1600 1610 1620
AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>
1630 1640 1650 1660
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg>
c___c___c___c___HUMAN IGG1 FC TAG__c___c___c___c___c___>

Figure 15E

```
1670          1680          1690          1700          1710
   *     *     *     *     *     *     *     *     *
GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>

         1720          1730          1740          1750
   *     *     *     *     *     *     *     *     *
AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>

1760          1770          1780          1790          1800
   *     *     *     *     *     *     *     *     *
AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>

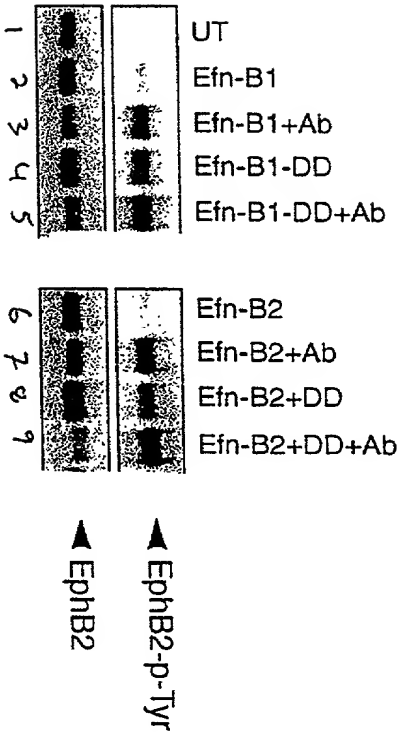
         1810          1820          1830          1840
   *     *     *     *     *     *     *     *     *
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>

1850          1860          1870          1880          1890
   *     *     *     *     *     *     *     *     *
TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>

         1900          1910          1920          1930
   *     *     *     *     *     *     *     *     *
GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>
___c___c___c___c____HUMAN IGG1 FC TAG__c___c___c___c___c___>

1940          1950          1960          1970
   *     *     *     *     *     *     *     *
CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>
___c___c___c___c__HUMAN IGG1 FC TAG____c___c___c___c___>
```

Figure 16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3
4
5
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Efn-B1-DD
Efn-B1-DD+Ab
6
7
8
9
Efn-B2
Efn-B2+Ab
Efn-B2+DD
Efn-B2+DD+Ab
EphB2
EphB2-p-Tyr

Figure 17

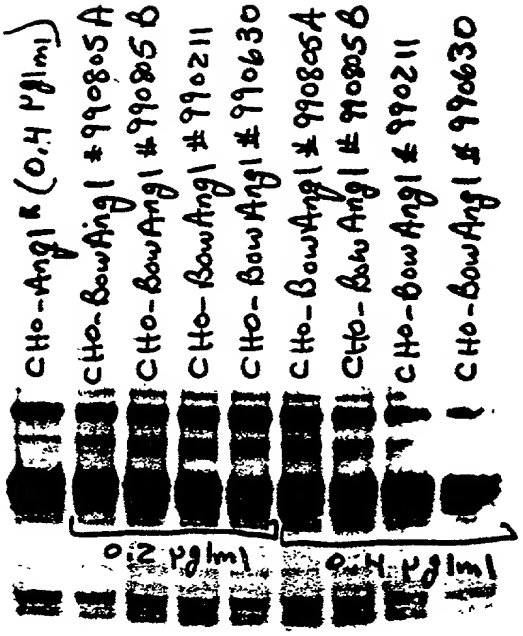
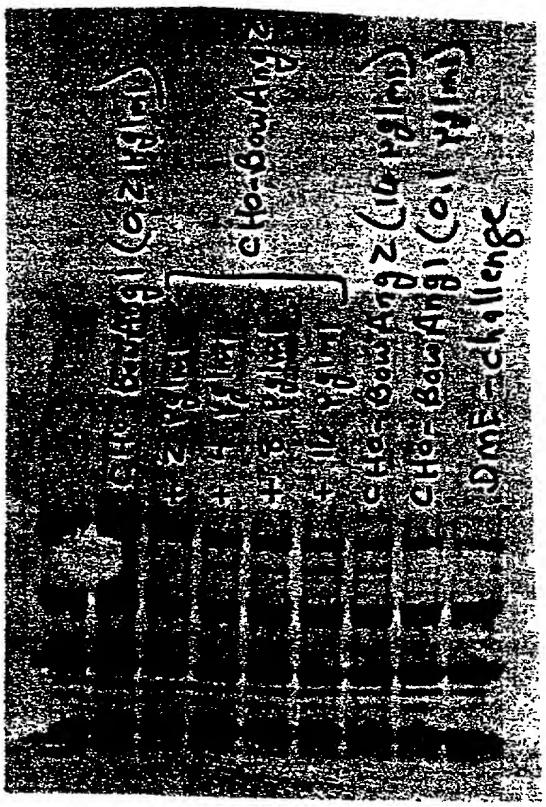

CHO-Angl (0.4 µg/ml)
CHO-BowAngl #990805A
CHO-BowAngl #990805B
CHO-BowAngl #990211
CHO-BowAngl #990630
CHO-BowAngl #990805A
CHO-BowAngl #990805B
CHO-BowAngl #990211
CHO-BowAngl #990630
0.2 µg/ml
0.4 µg/ml
1 2 3 4 5 6 7 8 9

Figure 18



1 2 3 4 5 6 7 8 9

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled **METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS**, USSN 09/868,677 filed June 20, 2001, which is the national stage filing of International Application No. PCT/US99/30900, filed December 23, 1999.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to in the oath or declaration.

I acknowledge the duty to disclose information of which I am aware that is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PCT/US99/30900 filed December 23, 1999

I hereby claim the benefit under Title 35, United States Code, §119(e) and 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States Application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) that occurred between the filing date of the prior application and the national or PCT international filing date of this application:

USSN 60/113,387 filed December 23, 1998

(3)

And I hereby appoint Joseph M. Sorrentino (Registration No. 32,598), Gail M. Kempler (Registration No. 32,143), and Linda O. Palladino (Registration No. 45,636), each of them my attorneys and agent, each with full power of substitution and revocation, to prosecute this application, to make alterations and

amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications that are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

Linda O. Palladino
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
Tel. (914-345-7400)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1200

Inventor: Samuel J. Davis SD

Signature: Samuel J. Davis Date: 9/28/01

Citizenship: United States of America

Residence: 332 W. 885th Street, #B2
New York, New York 10024 NY
United States of America

Post Office Address: same as residence

2-00

Att. Docket No. REG 670A-US
National Stage Filing of
Int'l File No. PCT/US99/30900
Declaration and Power of Attorney

Inventor: Nicholas W. Gale

Signature: Nicholas W. Gale Date: 9/28/01

Citizenship: United States of America

Residence: 177 White Plains Road, Apt. 46V
Tarrytown, New York 10591 NY.
United States of America

Post Office Address: same as residence

2-00

Inventor: George D. Yancopoulos

Signature: George D. Yancopoulos Date: 9/28/01

Citizenship: United States of America

Residence: 1519 Baptist Church Road
Yorktown Heights, New York 10598 NY.
United States of America

Post Office Address: same as residence

Att. Docket No. REG 670A-US
National Stage Filing of
Int'l File No. PCT/US99/30900
Declaration and Power of Attorney

4-02

Inventor: Neil Stahl

Signature: Neil Stahl Date: 9/28/01

Citizenship: United States of America

Residence: 48 Kent Shore Drive
Carmel, New York 10512 NY
United States of America

Post Office Address: same as residence

SEQUENCE LISTING

<110> Samuel Davis, Nicholas W. Gale, George D. Yancopoulos, and Neil Stahl

<120> METHOD OF ENHANCING THE BIOLOGICAL ACTIVITY OF LIGANDS

<130> REG 670-A-US

<140> USSN 09/868,677
<141> 2001-10-01

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<151> 1999-12-23

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Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15

gac tgt gca gat gta tat caa gct ggt ttt aat aaa agt gga atc tac       96
Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr
            20                  25                  30

act att tat att aat aat atg cca gaa ccc aaa aag gtg ttt tgc aat      144
Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn
        35                  40                  45

atg gat gtc aat ggg gga ggt tgg act gta ata caa cat cgt gaa gat      192
Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp
    50                  55                  60

gga agt cta gat ttc caa aga ggc tgg aag gaa tat aaa atg ggt ttt      240
Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe
65                  70                  75                  80

gga aat ccc tcc ggt gaa tat tgg ctg ggg aat gag ttt att ttt gcc      288
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala
                85                  90                  95

att acc agt cag agg cag tac atg cta aga att gag tta atg gac tgg      336
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp
            100                 105                 110
```

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gaa ggg aac cga gcc tat tca cag tat gac aga ttc cac ata gga aat      384
Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn
        115                 120                 125

gaa aag caa aac tat agg ttg tat tta aaa ggt cac act ggg aca gca      432
Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala
    130                 135                 140

gga aaa cag agc agc ctg atc tta cac ggt gct gat ttc agc act aaa      480
Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys
145                 150                 155                 160

gat gct gat aat gac aac tgt atg tgc aaa tgt gcc ctc atg tta aca      528
Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr
                165                 170                 175

gga gga tgg tgg ttt gat gct tgt ggc ccc tcc aat cta aat gga atg      576
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190

ttc tat act gcg gga caa aac cat gga aaa ctg aat ggg ata aag tgg      624
Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp
        195                 200                 205

cac tac ttc aaa ggg ccc agt tac tcc tta cgt tcc aca act atg atg      672
His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met
    210                 215                 220

att cga cct tta gat ttt ggc ccc gcg cct ttt aga gac tgt gca gat      720
Ile Arg Pro Leu Asp Phe Gly Pro Ala Pro Phe Arg Asp Cys Ala Asp
225                 230                 235                 240

gta tat caa gct ggt ttt aat aaa agt gga atc tac act att tat att      768
Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile
                245                 250                 255

aat aat atg cca gaa ccc aaa aag gtg ttt tgc aat atg gat gtc aat      816
Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn
            260                 265                 270

ggg gga ggt tgg act gta ata caa cat cgt gaa gat gga agt cta gat      864
Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp
        275                 280                 285

ttc caa aga ggc tgg aag gaa tat aaa atg ggt ttt gga aat ccc tcc      912
Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser
    290                 295                 300

ggt gaa tat tgg ctg ggg aat gag ttt att ttt gcc att acc agt cag      960
Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln
305                 310                 315                 320

agg cag tac atg cta aga att gag tta atg gac tgg gaa ggg aac cga     1008
Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg
                325                 330                 335

gcc tat tca cag tat gac aga ttc cac ata gga aat gaa aag caa aac     1056
Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn
            340                 345                 350
```

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tat agg ttg tat tta aaa ggt cac act ggg aca gca gga aaa cag agc      1104
Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser
        355                 360                 365

agc ctg atc tta cac ggt gct gat ttc agc act aaa gat gct gat aat      1152
Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn
    370                 375                 380

gac aac tgt atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg tgg      1200
Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp
385                 390                 395                 400

ttt gat gct tgt ggc ccc tcc aat cta aat gga atg ttc tat act gcg      1248
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala
                405                 410                 415

gga caa aac cat gga aaa ctg aat ggg ata aag tgg cac tac ttc aaa      1296
Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys
            420                 425                 430

ggg ccc agt tac tcc tta cgt tcc aca act atg atg att cga cct tta      1344
Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu
        435                 440                 445

gat ttt gga ccg ggc gag ccc aaa tct tgt gac aaa act cac aca tgc      1392
Asp Phe Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
    450                 455                 460

cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc      1440
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
465                 470                 475                 480

ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag      1488
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                485                 490                 495

gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag      1536
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            500                 505                 510

ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag      1584
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        515                 520                 525

ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc      1632
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    530                 535                 540

acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag      1680
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
545                 550                 555                 560

gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa      1728
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                565                 570                 575

gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc      1776
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            580                 585                 590

cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa      1824
```

```
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        595                 600                 605

ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag      1872
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    610                 615                 620

ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc      1920
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
625                 630                 635                 640

tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag      1968
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                645                 650                 655

cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac      2016
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            660                 665                 670

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga              2058
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        675                 680                 685
```

<210> 2
<211> 685
<212> PRT
<213> Homo sapiens

<400> 2

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Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15
Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr
            20                  25                  30
Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn
        35                  40                  45
Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp
    50                  55                  60
Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe
65                  70                  75                  80
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala
                85                  90                  95
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp
            100                 105                 110
Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn
        115                 120                 125
Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala
    130                 135                 140
Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys
145                 150                 155                 160
Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr
                165                 170                 175
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190
Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp
        195                 200                 205
His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met
    210                 215                 220
Ile Arg Pro Leu Asp Phe Gly Pro Ala Pro Phe Arg Asp Cys Ala Asp
225                 230                 235                 240
Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile
```

245 250 255

Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn
260 265 270

Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp
275 280 285

Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser
290 295 300

Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln
305 310 315 320

Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg
325 330 335

Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn
340 345 350

Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser
355 360 365

Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn
370 375 380

Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp
385 390 395 400

Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala
405 410 415

Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys
420 425 430

Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu
435 440 445

Asp Phe Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
450 455 460

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
465 470 475 480

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
485 490 495

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
500 505 510

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
515 520 525

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
530 535 540

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
545 550 555 560

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
565 570 575

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
580 585 590

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
595 600 605

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
610 615 620

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
625 630 635 640

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
645 650 655

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
660 665 670

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
675 680 685

<210> 3
<211> 2061
<212> DNA
<213> Homo sapiens

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<220>

<221> CDS
<222> (1)...(2058)

<400> 3
atg tct gca ctt ctg atc cta gct ctt gtt gga gct gca gtt gct aga        48
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
1               5                   10                  15

gac tgt gct gaa gta ttc aaa tca gga cac acc aca aat ggc atc tac        96
Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr
            20                  25                  30

acg tta aca ttc cct aat tct aca gaa gag atc aag gcc tac tgt gac       144
Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp
        35                  40                  45

atg gaa gct gga gga ggc ggg tgg aca att att cag cga cgt gag gat       192
Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp
    50                  55                  60

ggc agc gtt gat ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt       240
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe
65                  70                  75                  80

ggt aac cct tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa       288
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln
                85                  90                  95

ctg act aat cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg       336
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp
            100                 105                 110

gaa ggg aat gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt       384
Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser
        115                 120                 125

gaa gaa ctc aat tat agg att cac ctt aaa gga ctt aca ggg aca gcc       432
Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala
    130                 135                 140

ggc aaa ata agc agc atc agc caa cca gga aat gat ttt agc aca aag       480
Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys
145                 150                 155                 160

gat gga gac aac gac aaa tgt att tgc aaa tgt tca caa atg cta aca       528
Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr
                165                 170                 175

gga ggc tgg tgg ttt gat gca tgt ggt cct tcc aac ttg aac gga atg       576
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190

tac tat cca cag agg cag aac aca aat aag ttc aac ggc att aaa tgg       624
Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp
        195                 200                 205

tac tac tgg aaa ggc tca ggc tat tcg ctc aag gcc aca acc atg atg       672
Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met
    210                 215                 220
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atc cga cca gca gat ttc ggg ggc ccc gcg cct ttc aga gac tgt gct      720
Ile Arg Pro Ala Asp Phe Gly Gly Pro Ala Pro Phe Arg Asp Cys Ala
225                 230                 235                 240

gaa gta ttc aaa tca gga cac acc aca aat ggc atc tac acg tta aca      768
Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr
                245                 250                 255

ttc cct aat tct aca gaa gag atc aag gcc tac tgt gac atg gaa gct      816
Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala
            260                 265                 270

gga gga ggc ggg tgg aca att att cag cga cgt gag gat ggc agc gtt      864
Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val
        275                 280                 285

gat ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt ggt aac cct      912
Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro
    290                 295                 300

tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa ctg act aat      960
Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn
305                 310                 315                 320

cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg gaa ggg aat     1008
Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn
                325                 330                 335

gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt gaa gaa ctc     1056
Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu
            340                 345                 350

aat tat agg att cac ctt aaa gga ctt aca ggg aca gcc ggc aaa ata     1104
Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile
        355                 360                 365

agc agc atc agc caa cca gga aat gat ttt agc aca aag gat gga gac     1152
Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp
    370                 375                 380

aac gac aaa tgt att tgc aaa tgt tca caa atg cta aca gga ggc tgg     1200
Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp
385                 390                 395                 400

tgg ttt gat gca tgt ggt cct tcc aac ttg aac gga atg tac tat cca     1248
Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro
                405                 410                 415

cag agg cag aac aca aat aag ttc aac ggc att aaa tgg tac tac tgg     1296
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp
            420                 425                 430

aaa ggc tca ggc tat tcg ctc aag gcc aca acc atg atg atc cga cca     1344
Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro
        435                 440                 445

gca gat ttc gga ccg ggc gag ccc aaa tct tgt gac aaa act cac aca     1392
Ala Asp Phe Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    450                 455                 460
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tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc      1440
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
465                 470                 475                 480

ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct      1488
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                485                 490                 495

gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc      1536
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
            500                 505                 510

aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca      1584
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        515                 520                 525

aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc      1632
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
    530                 535                 540

ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc      1680
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
545                 550                 555                 560

aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc      1728
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                565                 570                 575

aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca      1776
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            580                 585                 590

tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc      1824
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        595                 600                 605

aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg      1872
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
    610                 615                 620

cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac      1920
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
625                 630                 635                 640

ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg      1968
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                645                 650                 655

cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac      2016
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
            660                 665                 670

aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa              2058
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        675                 680                 685

tga                                                                 2061
```

<210> 4
<211> 686
<212> PRT

<213> Homo sapiens

<400> 4

```
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15
Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr
            20                  25                  30
Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp
        35                  40                  45
Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp
    50                  55                  60
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe
65                  70                  75                  80
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln
                85                  90                  95
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp
            100                 105                 110
Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser
        115                 120                 125
Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala
    130                 135                 140
Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys
145                 150                 155                 160
Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr
                165                 170                 175
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190
Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp
        195                 200                 205
Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met
    210                 215                 220
Ile Arg Pro Ala Asp Phe Gly Gly Pro Ala Pro Phe Arg Asp Cys Ala
225                 230                 235                 240
Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr
                245                 250                 255
Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala
            260                 265                 270
Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val
        275                 280                 285
Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro
    290                 295                 300
Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn
305                 310                 315                 320
Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn
                325                 330                 335
Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu
            340                 345                 350
Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile
        355                 360                 365
Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp
    370                 375                 380
Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp
385                 390                 395                 400
Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro
                405                 410                 415
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp
            420                 425                 430
Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro
        435                 440                 445
Ala Asp Phe Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    450                 455                 460
```

```
Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
465                 470                 475                 480
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                485                 490                 495
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
            500                 505                 510
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        515                 520                 525
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
    530                 535                 540
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
545                 550                 555                 560
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                565                 570                 575
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            580                 585                 590
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        595                 600                 605
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
    610                 615                 620
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
625                 630                 635                 640
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                645                 650                 655
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
            660                 665                 670
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        675                 680                 685
```

<210> 5
<211> 2067
<212> DNA
<213> Homo sapiens

<220>

<221> CDS
<222> (1)...(2064)

<400> 5

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atg tct gca ctt ctg atc cta gct ctt gtt gga gct gca gtt gct aga      48
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15

gac tgt gca gat gta tat caa gct ggt ttt aat aaa agt gga atc tac      96
Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr
            20                  25                  30

act att tat att aat aat atg cca gaa ccc aaa aag gtg ttt tgc aat     144
Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn
        35                  40                  45

atg gat gtc aat ggg gga ggt tgg act gta ata caa cat cgt gaa gat     192
Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp
    50                  55                  60

gga agt cta gat ttc caa aga ggc tgg aag gaa tat aaa atg ggt ttt     240
Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe
65                  70                  75                  80

gga aat ccc tcc ggt gaa tat tgg ctg ggg aat gag ttt att ttt gcc     288
```

```
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala
                85                  90                  95

att acc agt cag agg cag tac atg cta aga att gag tta atg gac tgg      336
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp
            100                 105                 110

gaa ggg aac cga gcc tat tca cag tat gac aga ttc cac ata gga aat      384
Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn
        115                 120                 125

gaa aag caa aac tat agg ttg tat tta aaa ggt cac act ggg aca gca      432
Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala
    130                 135                 140

gga aaa cag agc agc ctg atc tta cac ggt gct gat ttc agc act aaa      480
Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys
145                 150                 155                 160

gat gct gat aat gac aac tgt atg tgc aaa tgt gcc ctc atg tta aca      528
Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr
                165                 170                 175

gga gga tgg tgg ttt gat gct tgt ggc ccc tcc aat cta aat gga atg      576
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190

ttc tat act gcg gga caa aac cat gga aaa ctg aat ggg ata aag tgg      624
Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp
        195                 200                 205

cac tac ttc aaa ggg cca agt tac tcc tta cgt tcc aca act atg atg      672
His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met
    210                 215                 220

att cga cct tta gat ttt ggc ccg ggc gag ccc aaa tct tgt gac aaa      720
Ile Arg Pro Leu Asp Phe Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys
225                 230                 235                 240

act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg      768
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
                245                 250                 255

tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc      816
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
            260                 265                 270

cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac      864
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
        275                 280                 285

cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat      912
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
    290                 295                 300

gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg      960
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305                 310                 315                 320

gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag     1008
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
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                325                 330                 335

tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa  1056
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
            340                 345                 350

acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc  1104
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
        355                 360                 365

ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc  1152
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
    370                 375                 380

tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag  1200
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385                 390                 395                 400

agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg  1248
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                405                 410                 415

gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag  1296
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
            420                 425                 430

agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag  1344
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
        435                 440                 445

gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt  1392
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
    450                 455                 460

aaa ggc ggt ggc ggt tct ggc gcg cct ttt aga gac tgt gca gat gta  1440
Lys Gly Gly Gly Gly Ser Gly Ala Pro Phe Arg Asp Cys Ala Asp Val
465                 470                 475                 480

tat caa gct ggt ttt aat aaa agt gga atc tac act att tat att aat  1488
Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn
                485                 490                 495

aat atg cca gaa ccc aaa aag gtg ttt tgc aat atg gat gtc aat ggg  1536
Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly
            500                 505                 510

gga ggt tgg act gta ata caa cat cgt gaa gat gga agt cta gat ttc  1584
Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe
        515                 520                 525

caa aga ggc tgg aag gaa tat aaa atg ggt ttt gga aat ccc tcc ggt  1632
Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly
    530                 535                 540

gaa tat tgg ctg ggg aat gag ttt att ttt gcc att acc agt cag agg  1680
Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg
545                 550                 555                 560

cag tac atg cta aga att gag tta atg gac tgg gaa ggg aac cga gcc  1728
Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala
                565                 570                 575
```

```
tat tca cag tat gac aga ttc cac ata gga aat gaa aag caa aac tat      1776
Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
            580                 585                 590

agg ttg tat tta aaa ggt cac act ggg aca gca gga aaa cag agc agc      1824
Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
        595                 600                 605

ctg atc tta cac ggt gct gat ttc agc act aaa gat gct gat aat gac      1872
Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
    610                 615                 620

aac tgt atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg tgg ttt      1920
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
625                 630                 635                 640

gat gct tgt ggc ccc tcc aat cta aat gga atg ttc tat act gcg gga      1968
Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly
                645                 650                 655

caa aac cat gga aaa ctg aat ggg ata aag tgg cac tac ttc aaa ggg      2016
Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly
            660                 665                 670

cca agt tac tcc tta cgt tcc aca act atg atg att cga cct tta gat      2064
Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp
        675                 680                 685

ttt                                                                 2067
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<210> 6
<211> 688
<212> PRT
<213> Homo sapiens

<400> 6

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Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15
Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr
            20                  25                  30
Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn
        35                  40                  45
Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp
    50                  55                  60
Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe
65                  70                  75                  80
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala
                85                  90                  95
Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp
            100                 105                 110
Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn
        115                 120                 125
Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala
    130                 135                 140
Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys
145                 150                 155                 160
Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr
                165                 170                 175
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190
```

Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp
195 200 205
His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met
210 215 220
Ile Arg Pro Leu Asp Phe Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys
225 230 235 240
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
245 250 255
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
260 265 270
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
275 280 285
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
290 295 300
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305 310 315 320
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
325 330 335
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
340 345 350
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
355 360 365
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
370 375 380
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385 390 395 400
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
405 410 415
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
420 425 430
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
435 440 445
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
450 455 460
Lys Gly Gly Gly Gly Ser Gly Ala Pro Phe Arg Asp Cys Ala Asp Val
465 470 475 480
Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn
485 490 495
Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly
500 505 510
Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe
515 520 525
Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly
530 535 540
Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg
545 550 555 560
Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala
565 570 575
Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
580 585 590
Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
595 600 605
Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
610 615 620
Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
625 630 635 640
Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly
645 650 655
Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly
660 665 670
Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp

675 680 685

<210> 7
<211> 2070
<212> DNA
<213> Homo sapiens

<220>

<221> CDS
<222> (1)...(2067)

<400> 7

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atg tct gca ctt ctg atc cta gct ctt gtt gga gct gca gtt gct aga        48
Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15

gac tgt gct gaa gta ttc aaa tca gga cac acc aca aat ggc atc tac        96
Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr
            20                  25                  30

acg tta aca ttc cct aat tct aca gaa gag atc aag gcc tac tgt gac       144
Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp
        35                  40                  45

atg gaa gct gga gga ggc ggg tgg aca att att cag cga cgt gag gat       192
Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp
    50                  55                  60

ggc agc gtt gat ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt       240
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe
65                  70                  75                  80

ggt aac cct tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa       288
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln
                85                  90                  95

ctg act aat cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg       336
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp
            100                 105                 110

gaa ggg aat gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt       384
Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser
        115                 120                 125

gaa gaa ctc aat tat agg att cac ctt aaa gga ctt aca ggg aca gcc       432
Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala
    130                 135                 140

ggc aaa ata agc agc atc agc caa cca gga aat gat ttt agc aca aag       480
Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys
145                 150                 155                 160

gat gga gac aac gac aaa tgt att tgc aaa tgt tca caa atg cta aca       528
Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr
                165                 170                 175

gga ggc tgg tgg ttt gat gca tgt ggt cct tcc aac ttg aac gga atg       576
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190
```

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tac tat cca cag agg cag aac aca aat aag ttc aac ggc att aaa tgg      624
Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp
        195                 200                 205

tac tac tgg aaa ggc tca ggc tat tcg ctc aag gcc aca acc atg atg      672
Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met
    210                 215                 220

atc cga cca gca gat ttc ggg ggc ccg ggc gag ccc aaa tct tgt gac      720
Ile Arg Pro Ala Asp Phe Gly Gly Pro Gly Glu Pro Lys Ser Cys Asp
225                 230                 235                 240

aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga      768
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
                245                 250                 255

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc      816
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            260                 265                 270

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa      864
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        275                 280                 285

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat      912
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt      960
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
305                 310                 315                 320

gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag     1008
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag     1056
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
            340                 345                 350

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac     1104
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        355                 360                 365

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg     1152
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
    370                 375                 380

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg     1200
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385                 390                 395                 400

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg     1248
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
                405                 410                 415

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac     1296
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
            420                 425                 430

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat     1344
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Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
        435                 440                 445

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg      1392
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
    450                 455                 460

ggt aaa ggc ggt ggc ggt tct ggc gcg cct aga gac tgt gct gaa gta      1440
Gly Lys Gly Gly Gly Gly Ser Gly Ala Pro Arg Asp Cys Ala Glu Val
465                 470                 475                 480

ttc aaa tca gga cac acc aca aat ggc atc tac acg tta aca ttc cct      1488
Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro
                485                 490                 495

aat tct aca gaa gag atc aag gcc tac tgt gac atg gaa gct gga gga      1536
Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly
            500                 505                 510

ggc ggg tgg aca att att cag cga cgt gag gat ggc agc gtt gat ttt      1584
Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe
        515                 520                 525

cag agg act tgg aaa gaa tat aaa gtg gga ttt ggt aac cct tca gga      1632
Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly
    530                 535                 540

gaa tat tgg ctg gga aat gag ttt gtt tcg caa ctg act aat cag caa      1680
Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln
545                 550                 555                 560

cgc tat gtg ctt aaa ata cac ctt aaa gac tgg gaa ggg aat gag gct      1728
Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala
                565                 570                 575

tac tca ttg tat gaa cat ttc tat ctc tca agt gaa gaa ctc aat tat      1776
Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr
            580                 585                 590

agg att cac ctt aaa gga ctt aca ggg aca gcc ggc aaa ata agc agc      1824
Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser
        595                 600                 605

atc agc caa cca gga aat gat ttt agc aca aag gat gga gac aac gac      1872
Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp
    610                 615                 620

aaa tgt att tgc aaa tgt tca caa atg cta aca gga ggc tgg tgg ttt      1920
Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe
625                 630                 635                 640

gat gca tgt ggt cct tcc aac ttg aac gga atg tac tat cca cag agg      1968
Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg
                645                 650                 655

cag aac aca aat aag ttc aac ggc att aaa tgg tac tac tgg aaa ggc      2016
Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly
            660                 665                 670

tca ggc tat tcg ctc aag gcc aca acc atg atg atc cga cca gca gat      2064
Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp
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675 680 685

ttc tga 2070
Phe

<210> 8
<211> 689
<212> PRT
<213> Homo sapiens

<400> 8

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Met Ser Ala Leu Leu Ile Leu Ala Leu Val Gly Ala Ala Val Ala Arg
 1               5                  10                  15
Asp Cys Ala Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr
            20                  25                  30
Thr Leu Thr Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp
        35                  40                  45
Met Glu Ala Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp
    50                  55                  60
Gly Ser Val Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe
65                  70                  75                  80
Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln
                85                  90                  95
Leu Thr Asn Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp
            100                 105                 110
Glu Gly Asn Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser
        115                 120                 125
Glu Glu Leu Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala
    130                 135                 140
Gly Lys Ile Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys
145                 150                 155                 160
Asp Gly Asp Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr
                165                 170                 175
Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met
            180                 185                 190
Tyr Tyr Pro Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp
        195                 200                 205
Tyr Tyr Trp Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met
    210                 215                 220
Ile Arg Pro Ala Asp Phe Gly Gly Pro Gly Glu Pro Lys Ser Cys Asp
225                 230                 235                 240
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
                245                 250                 255
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            260                 265                 270
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        275                 280                 285
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
305                 310                 315                 320
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
            340                 345                 350
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        355                 360                 365
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
    370                 375                 380
```

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Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385                 390                 395                 400
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
                405                 410                 415
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
            420                 425                 430
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
        435                 440                 445
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
    450                 455                 460
Gly Lys Gly Gly Gly Gly Ser Gly Ala Pro Arg Asp Cys Ala Glu Val
465                 470                 475                 480
Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro
                485                 490                 495
Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly
            500                 505                 510
Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe
        515                 520                 525
Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly
    530                 535                 540
Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln
545                 550                 555                 560
Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala
                565                 570                 575
Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr
            580                 585                 590
Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser
        595                 600                 605
Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp
    610                 615                 620
Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe
625                 630                 635                 640
Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg
                645                 650                 655
Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly
            660                 665                 670
Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp
        675                 680                 685
Phe
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<210> 9
<211> 2052
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(2049)

<400> 9

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atg gct cgg cct ggg cag cgt tgg ctc ggc aag tgg ctt gtg gcg atg        48
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
 1               5                  10                  15

gtc gtg tgg gcg ctg tgc cgg ctc gcc aca ccg ctg gcc aag aac ctg        96
Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
            20                  25                  30

gag ccc gta tcc tgg agc tcc ctc aac ccc aag ttc ctg agt ggg aag       144
Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
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                35                  40                  45

ggc ttg gtg atc tat ccg aaa att gga gac aag ctg gac atc atc tgc      192
Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
     50                  55                  60

ccc cga gca gaa gca ggg cgg ccc tat gag tac tac aag ctg tac ctg      240
Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
 65                  70                  75                  80

gtg cgg cct gag cag gca gct gcc tgt agc aca gtt ctc gac ccc aac      288
Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
                 85                  90                  95

gtg ttg gtc acc tgc aat agg cca gag cag gaa ata cgc ttt acc atc      336
Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
            100                 105                 110

aag ttc cag gag ttc agc ccc aac tac atg ggc ctg gag ttc aag aag      384
Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
        115                 120                 125

cac cat gat tac tac att acc tca aca tcc aat gga agc ctg gag ggg      432
His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
    130                 135                 140

ctg gaa aac cgg gag ggc ggt gtg tgc cgc aca cgc acc atg aag atc      480
Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145                 150                 155                 160

atc atg aag gtt ggg caa gat ccc aat gct gtg acg cct gag cag ctg      528
Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
                165                 170                 175

act acc agc agg ccc agc aag gag gca gac aac act gtc aag atg gcc      576
Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala
            180                 185                 190

aca cag gcc cct ggt agt cgg ggc tcc ctg ggt gac tct gat ggc aag      624
Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
        195                 200                 205

cat gag act gtg aac cag gaa gag aag agt ggc cca ggt gca agt ggg      672
His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
    210                 215                 220

ggc agc agc ggg gac cct gat ggc ttc ttc aac tcc aag ggc ccg ggt      720
Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys Gly Pro Gly
225                 230                 235                 240

aag aac ctg gag ccc gta tcc tgg agc tcc ctc aac ccc aag ttc ctg      768
Lys Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu
                245                 250                 255

agt ggg aag ggc ttg gtg atc tat ccg aaa att gga gac aag ctg gac      816
Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp
            260                 265                 270

atc atc tgc ccc cga gca gaa gca ggg cgg ccc tat gag tac tac aag      864
Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys
        275                 280                 285
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ctg tac ctg gtg cgg cct gag cag gca gct gcc tgt agc aca gtt ctc      912
Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Cys Ser Thr Val Leu
    290                 295                 300

gac ccc aac gtg ttg gtc acc tgc aat agg cca gag cag gaa ata cgc      960
Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg
305                 310                 315                 320

ttt acc atc aag ttc cag gag ttc agc ccc aac tac atg ggc ctg gag     1008
Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu
                325                 330                 335

ttc aag aag cac cat gat tac tac att acc tca aca tcc aat gga agc     1056
Phe Lys Lys His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser
            340                 345                 350

ctg gag ggg ctg gaa aac cgg gag ggc ggt gtg tgc cgc aca cgc acc     1104
Leu Glu Gly Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr
        355                 360                 365

atg aag atc atc atg aag gtt ggg caa gat ccc aat gct gtg acg cct     1152
Met Lys Ile Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro
    370                 375                 380

gag cag ctg act acc agc agg ccc agc aag gag gca gac aac act gtc     1200
Glu Gln Leu Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val
385                 390                 395                 400

aag atg gcc aca cag gcc cct ggt agt cgg ggc tcc ctg ggt gac tct     1248
Lys Met Ala Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser
                405                 410                 415

gat ggc aag cat gag act gtg aac cag gaa gag aag agt ggc cca ggt     1296
Asp Gly Lys His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly
            420                 425                 430

gca agt ggg ggc agc agc ggg gac cct gat ggc ttc ttc aac tcc aaa     1344
Ala Ser Gly Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys
        435                 440                 445

ggc ccg ggc gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg     1392
Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    450                 455                 460

tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc     1440
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
465                 470                 475                 480

cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca     1488
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                485                 490                 495

tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac     1536
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            500                 505                 510

tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg     1584
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        515                 520                 525
```

```
gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc      1632
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    530                 535                 540

ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc      1680
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
545                 550                 555                 560

aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa      1728
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                565                 570                 575

ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat      1776
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
            580                 585                 590

gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc      1824
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        595                 600                 605

tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag      1872
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    610                 615                 620

aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc      1920
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
625                 630                 635                 640

ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg      1968
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                645                 650                 655

aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac      2016
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            660                 665                 670

acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga                      2052
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        675                 680
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<210> 10
<211> 683
<212> PRT
<213> Homo sapiens

<400> 10

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Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
 1               5                  10                  15
Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
            20                  25                  30
Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
        35                  40                  45
Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
    50                  55                  60
Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
65                  70                  75                  80
Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
                85                  90                  95
Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
            100                 105                 110
```

Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
115 120 125
His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
130 135 140
Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145 150 155 160
Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
165 170 175
Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala
180 185 190
Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
195 200 205
His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
210 215 220
Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys Gly Pro Gly
225 230 235 240
Lys Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu
245 250 255
Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp
260 265 270
Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys
275 280 285
Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu
290 295 300
Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg
305 310 315 320
Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu
325 330 335
Phe Lys Lys His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser
340 345 350
Leu Glu Gly Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr
355 360 365
Met Lys Ile Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro
370 375 380
Glu Gln Leu Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val
385 390 395 400
Lys Met Ala Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser
405 410 415
Asp Gly Lys His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly
420 425 430
Ala Ser Gly Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys
435 440 445
Gly Pro Gly Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
450 455 460
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
465 470 475 480
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
485 490 495
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
500 505 510
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
515 520 525
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
530 535 540
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
545 550 555 560
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
565 570 575
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
580 585 590
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe

```
        595                 600                 605
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    610                 615                 620
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
625                 630                 635                 640
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                645                 650                 655
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            660                 665                 670
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        675                 680
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<210> 11
<211> 1977
<212> DNA
<213> Artificial Sequence

<220>
<223> Ephrin-B2-Ephrin-B2-Fc

<221> CDS
<222> (1)...(1974)

<400> 11

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atg gcc atg gcc cgg tcc agg agg gac tct gtg tgg aag tac tgt tgg      48
Met Ala Met Ala Arg Ser Arg Arg Asp Ser Val Trp Lys Tyr Cys Trp
 1               5                  10                  15

gga ctt ttg atg gtt ttg tgc aga act gcg atc tcc aga tcg ata gtt      96
Gly Leu Leu Met Val Leu Cys Arg Thr Ala Ile Ser Arg Ser Ile Val
            20                  25                  30

tta gag cct atc tac tgg aat tcc tcg aac tcc aaa ttt cta ccc gga     144
Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn Ser Lys Phe Leu Pro Gly
        35                  40                  45

caa ggc ctg gta cta tac cca cag ata gga gac aaa ttg gat att att     192
Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly Asp Lys Leu Asp Ile Ile
    50                  55                  60

tgc ccc aaa gtg gac tct aaa act gtt ggc cag tat gaa tat tat aaa     240
Cys Pro Lys Val Asp Ser Lys Thr Val Gly Gln Tyr Glu Tyr Tyr Lys
65                  70                  75                  80

gtt tat atg gtt gat aaa gac caa gca gac aga tgc aca att aag aag     288
Val Tyr Met Val Asp Lys Asp Gln Ala Asp Arg Cys Thr Ile Lys Lys
                85                  90                  95

gag aat acc ccg ctg ctc aac tgt gcc aga cca gac caa gat gtg aaa     336
Glu Asn Thr Pro Leu Leu Asn Cys Ala Arg Pro Asp Gln Asp Val Lys
            100                 105                 110

ttc acc atc aag ttt caa gaa ttc agc cct aac ctc tgg ggt cta gaa     384
Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Leu Trp Gly Leu Glu
        115                 120                 125

ttt cag aag aac aaa gat tac tac att ata tct aca tca aat ggg tct     432
Phe Gln Lys Asn Lys Asp Tyr Tyr Ile Ile Ser Thr Ser Asn Gly Ser
    130                 135                 140

ttg gag ggc ctg gat aac cag gag gga ggg gtg tgc cag aca aga gcc     480
```

```
Leu Glu Gly Leu Asp Asn Gln Glu Gly Gly Val Cys Gln Thr Arg Ala
145                 150                 155                 160

atg aag atc ctc atg aaa gtt gga caa gat gca agt tct gct gga tca      528
Met Lys Ile Leu Met Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser
                165                 170                 175

gcc agg aat cac ggt cca aca aga cgt cca gag cta gaa gct ggt aca      576
Ala Arg Asn His Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr
            180                 185                 190

aat ggg aga agt tca aca aca agt ccc ttt gtg aag cca aat cca ggt      624
Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro Gly
        195                 200                 205

tct agc acc gat ggc aac agc gcg ggg cat tcc ggg aac aat ctc ctg      672
Ser Ser Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn Leu Leu
    210                 215                 220

ggg ggc ccg gga ata gtt tta gag cct atc tac tgg aat tcc tcg aac      720
Gly Gly Pro Gly Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn
225                 230                 235                 240

tcc aaa ttt cta ccc gga caa ggc ctg gta cta tac cca cag ata gga      768
Ser Lys Phe Leu Pro Gly Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly
                245                 250                 255

gac aaa ttg gat att att tgc ccc aaa gtg gac tct aaa act gtt ggc      816
Asp Lys Leu Asp Ile Ile Cys Pro Lys Val Asp Ser Lys Thr Val Gly
            260                 265                 270

cag tat gaa tat tat aaa gtt tat atg gtt gat aaa gac caa gca gac      864
Gln Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp Gln Ala Asp
        275                 280                 285

aga tgc aca att aag aag gag aat acc ccg ctg ctc aac tgt gcc aga      912
Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu Asn Cys Ala Arg
    290                 295                 300

cca gac caa gat gtg aaa ttc acc atc aag ttt caa gaa ttc agc cct      960
Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro
305                 310                 315                 320

aac ctc tgg ggt cta gaa ttt cag aag aac aaa gat tac tac att ata     1008
Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn Lys Asp Tyr Tyr Ile Ile
                325                 330                 335

tct aca tca aat ggg tct ttg gag ggc ctg gat aac cag gag gga ggg     1056
Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Asp Asn Gln Glu Gly Gly
            340                 345                 350

gtg tgc cag aca aga gcc atg aag atc ctc atg aaa gtt gga caa gat     1104
Val Cys Gln Thr Arg Ala Met Lys Ile Leu Met Lys Val Gly Gln Asp
        355                 360                 365

gca agt tct gct gga tca gcc agg aat cac ggt cca aca aga cgc cca     1152
Ala Ser Ser Ala Gly Ser Ala Arg Asn His Gly Pro Thr Arg Arg Pro
    370                 375                 380

gag cta gaa gct ggt aca aat ggg aga agt tca aca aca agt ccc ttt     1200
Glu Leu Glu Ala Gly Thr Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe
```

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385                 390                 395                 400

gtg aag cca aat cca ggt tct agc acc gat ggc aac agc gcg ggg cat    1248
Val Lys Pro Asn Pro Gly Ser Ser Thr Asp Gly Asn Ser Ala Gly His
                405                 410                 415

tcc ggg aac aat ctc ctg ggg ggc ccg ggc gag ccc aaa tct tgt gac    1296
Ser Gly Asn Asn Leu Leu Gly Gly Pro Gly Glu Pro Lys Ser Cys Asp
            420                 425                 430

aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga    1344
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
        435                 440                 445

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc    1392
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    450                 455                 460

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa    1440
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
465                 470                 475                 480

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat    1488
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                485                 490                 495

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt    1536
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
            500                 505                 510

gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag    1584
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
        515                 520                 525

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag    1632
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
    530                 535                 540

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac    1680
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
545                 550                 555                 560

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg    1728
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
                565                 570                 575

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg    1776
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            580                 585                 590

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg    1824
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
        595                 600                 605

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac    1872
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    610                 615                 620

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat    1920
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
625                 630                 635                 640
```

```
gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg      1968
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                645                 650                 655

ggt aaa tga                                                          1977
Gly Lys
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<210> 12
<211> 658
<212> PRT
<213> Artificial Sequence

<220>

<223> Homo Sapier Fusion Protein

<400> 12

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 1               5                  10                  15
Gly Leu Leu Met Val Leu Cys Arg Thr Ala Ile Ser Arg Ser Ile Val
            20                  25                  30
Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn Ser Lys Phe Leu Pro Gly
        35                  40                  45
Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly Asp Lys Leu Asp Ile Ile
    50                  55                  60
Cys Pro Lys Val Asp Ser Lys Thr Val Gly Gln Tyr Glu Tyr Tyr Lys
65                  70                  75                  80
Val Tyr Met Val Asp Lys Asp Gln Ala Asp Arg Cys Thr Ile Lys Lys
                85                  90                  95
Glu Asn Thr Pro Leu Leu Asn Cys Ala Arg Pro Asp Gln Asp Val Lys
            100                 105                 110
Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Leu Trp Gly Leu Glu
        115                 120                 125
Phe Gln Lys Asn Lys Asp Tyr Tyr Ile Ile Ser Thr Ser Asn Gly Ser
    130                 135                 140
Leu Glu Gly Leu Asp Asn Gln Glu Gly Gly Val Cys Gln Thr Arg Ala
145                 150                 155                 160
Met Lys Ile Leu Met Lys Val Gly Gln Asp Ala Ser Ser Ala Gly Ser
                165                 170                 175
Ala Arg Asn His Gly Pro Thr Arg Arg Pro Glu Leu Glu Ala Gly Thr
            180                 185                 190
Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe Val Lys Pro Asn Pro Gly
        195                 200                 205
Ser Ser Thr Asp Gly Asn Ser Ala Gly His Ser Gly Asn Asn Leu Leu
    210                 215                 220
Gly Gly Pro Gly Ile Val Leu Glu Pro Ile Tyr Trp Asn Ser Ser Asn
225                 230                 235                 240
Ser Lys Phe Leu Pro Gly Gln Gly Leu Val Leu Tyr Pro Gln Ile Gly
                245                 250                 255
Asp Lys Leu Asp Ile Ile Cys Pro Lys Val Asp Ser Lys Thr Val Gly
            260                 265                 270
Gln Tyr Glu Tyr Tyr Lys Val Tyr Met Val Asp Lys Asp Gln Ala Asp
        275                 280                 285
Arg Cys Thr Ile Lys Lys Glu Asn Thr Pro Leu Leu Asn Cys Ala Arg
    290                 295                 300
Pro Asp Gln Asp Val Lys Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro
305                 310                 315                 320
Asn Leu Trp Gly Leu Glu Phe Gln Lys Asn Lys Asp Tyr Tyr Ile Ile
```

```
                325                 330                 335
Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Asp Asn Gln Glu Gly Gly
            340                 345                 350
Val Cys Gln Thr Arg Ala Met Lys Ile Leu Met Lys Val Gly Gln Asp
        355                 360                 365
Ala Ser Ser Ala Gly Ser Ala Arg Asn His Gly Pro Thr Arg Arg Pro
    370                 375                 380
Glu Leu Glu Ala Gly Thr Asn Gly Arg Ser Ser Thr Thr Ser Pro Phe
385                 390                 395                 400
Val Lys Pro Asn Pro Gly Ser Ser Thr Asp Gly Asn Ser Ala Gly His
                405                 410                 415
Ser Gly Asn Asn Leu Leu Gly Gly Pro Gly Glu Pro Lys Ser Cys Asp
            420                 425                 430
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
        435                 440                 445
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    450                 455                 460
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
465                 470                 475                 480
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                485                 490                 495
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
            500                 505                 510
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
        515                 520                 525
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
    530                 535                 540
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
545                 550                 555                 560
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
                565                 570                 575
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            580                 585                 590
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
        595                 600                 605
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    610                 615                 620
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
625                 630                 635                 640
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                645                 650                 655
Gly Lys
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<210> 13
<211> 4
<212> PRT
<213> Homo sapiens

<400> 13
Gly Pro Ala Pro
1

<210> 14
<211> 5
<212> PRT
<213> Homo sapiens

<400> 14
Gly Gly Pro Ala Pro
1   5

<210> 15
<211> 8
<212> PRT
<213> Homo sapiens

<400> 15
Gly Gly Gly Gly Ser Gly Ala Pro
1 5

<210> 16
<211> 4
<212> PRT
<213> Homo sapiens

<400> 16
Gly Gly Pro Gly
1

<210> 17
<211> 5
<212> PRT
<213> Homo sapiens

<400> 17
Arg Asp Cys Ala Asp
1 5

<210> 18
<211> 5
<212> PRT
<213> Homo sapiens

<400> 18
Arg Asp Cys Ala Glu
1 5

<210> 19
<211> 5
<212> PRT
<213> Homo sapiens

<400> 19
Asp Cys Ala Glu Val
1 5